Used 3

09/06

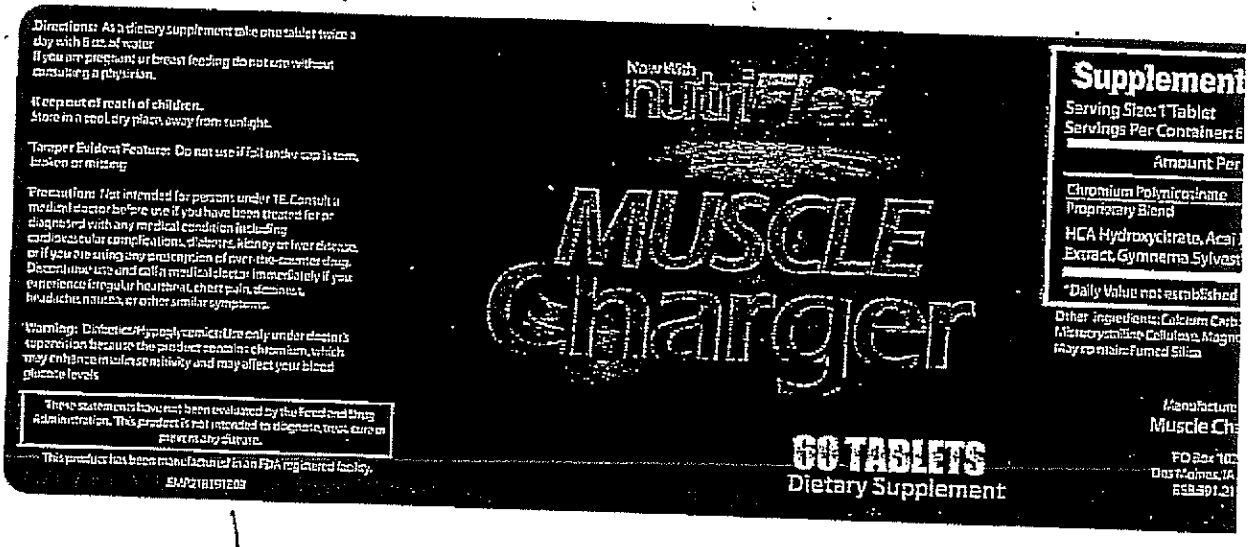
LABEL CONTROL RECEIVER

DATE RECEIVED 11/5/10 V

QUANTITY RECEIVED

CUSTOMER Just Thyroid Medicine COPY

PRINTER G



next printing change to SMP 2339 121709

Insert 3

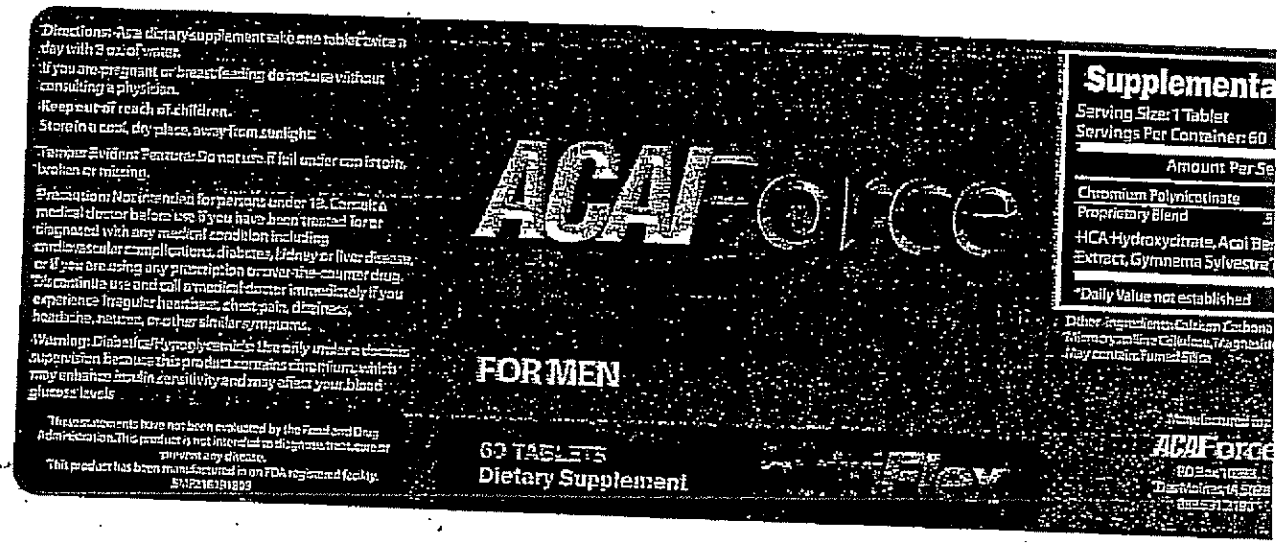
09/06

LABEL CONTROL RECEIVER

COPY

DATE RECEIVED 10/5/09 QUANTITY RECEIV

CUSTOMER Quest Think Media PRINTER



This product ---------

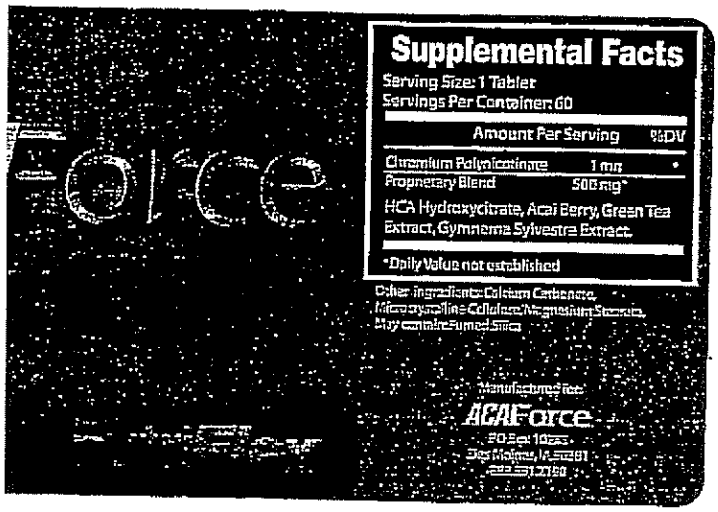
is outside of FDA statement by

9/06

ITROL RECEIVER

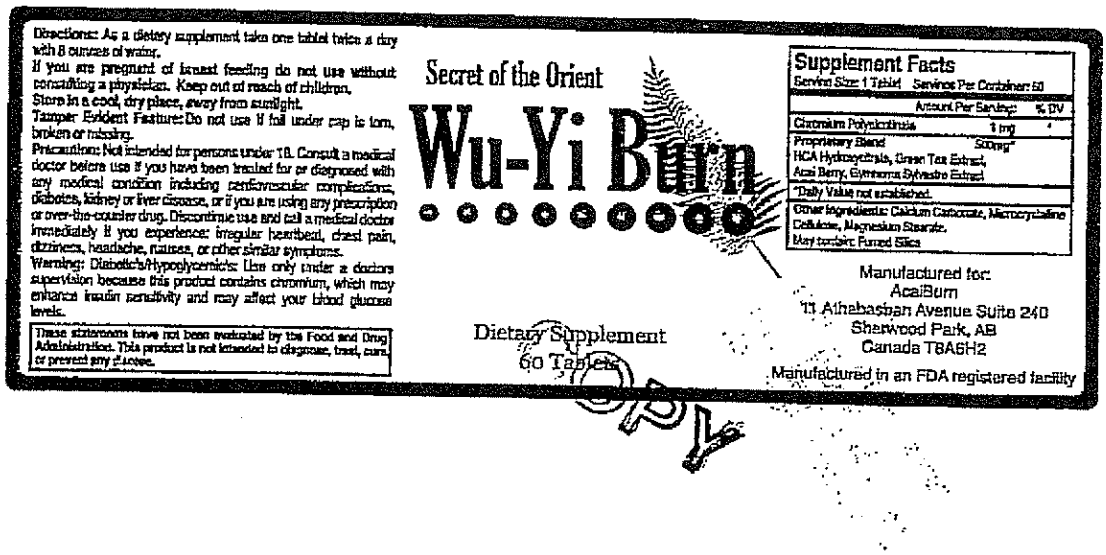
QUANTITY RECEIVED 1200

PRINTER



8-20-08

Size: 2.25 x 6.313
Colors: 2617 purple, magenta, 371 green, black



CONFIDENTIAL

NUTRI FTC 000007

NUTRICAP LABS

ph: 800.494.9154 ext. 4 • fax: 631.777.7107 • robertmoretz@nutricaplabs.com
200 Smith Street • Farmingdale, New York 11735 • www.nutricaplabs.com

date: XX.XX.XX Rev XX.XX.XX designer(s): XXXXX

file name: .ai

finished size: 2-1/4" x 6-5/8" 150cc | images: hi-res ☐ fpo ☐

this file is saved in adobe illustrator format
slug fonts: Helvetica medium | bold

printing colors: PANTONE XXXX, PANTONE XXXX, PANTONE XXXX, PANTONE XXXX, PANTONE XXXX, PANTONE XXXX | do not print: no line marks | stock: XXXXX LABEL

ATTENTION!

PLEASE READ CAREFULLY. BY SIGNING THE GRAPHICS PROJECT, YOU ARE RESPONSIBLE FOR THE REVIEWING THE FOLLOWING JOB ASSIGNED

GRAPHICS REVISIONS CHECK

Make sure the following is checked off before signing.

- ☐ Corrections made
- ☐ Spell check
- ☐ Check Colors
- ☐ Bar Code
- ☐ TM's and ® On Logos
- ☐ Signatures
- ☐ Current Sup facts w/signed off by Lab and Graphics attached
- ☐ Proof Read

Designer X ______ date

2nd Review X ______ date

X ______ date

______ Joy Provenzano - FINAL APPROVAL

______ Nutricap Account Executive - SPELL CHECK/PROOFREAD

______ Nutricap Account Executive - SUPPLEMENT FACTS/INGREDIENTS/DIRECTIONS

______ Art Director - SUPPLEMENT FACTS/INGREDIENTS/DIRECTIONS

______ Nutricap Account Executive - BARCODE

______ Art Director - TEMPLATE, GRAPHICS, SPELL CHECK, FINAL

NUTRICAP REVISIONS CHECK

Make sure the following is checked off before signing.

- ☐ Corrections made
- ☐ Spell check
- ☐ Check Colors
- ☐ Bar Code
- ☐ TM's and ® On Logos
- ☐ Signatures
- ☐ Current Sup facts w/signed off by Lab and Graphics attaced
- ☐ Proof Read
- ☐ Check product amounts on front panel
- ☐ Check product amounts in SF box

Acct. Exc X ______ date

2nd Review X ______ date

X ______ date

ELSEVIER

Physiology & Behavior 71 (2000) 87–94

PHYSIOLOGY & BEHAVIOR

Effects of (−)-hydroxycitric acid on appetitive variables

Richard D. Mattes*, Leslie Bormann

Department of Foods and Nutrition, Purdue University, Stone Hall, West Lafayette, IN 47907-1264, USA

Received 25 January 2000; received in revised form 18 April 2000; accepted 25 May 2000

Abstract

(−)-Hydroxycitric acid (HCA) reportedly promotes weight loss, in part, through suppression of hunger. However, this mechanism has never been evaluated in humans in a controlled study. Eighty-nine mildly overweight females were prescribed 5020-kJ diets for 12 weeks as part of a double-blind, placebo-controlled parallel group study. Forty-two participants ingested 400-mg caplets of *Garcinia cambogia* 30–60 min prior to meals for a total dose of 2.4 g/day (1.2 g/day HCA). Forty-seven participants ingested matched placebos. Weight and body composition were assessed at baseline and every other week for 12 weeks. Food intake and appetitive variables were assessed at baseline and monthly for 12 weeks. Both groups lost body weight with the active group achieving a significantly greater reduction (3.7±3.1 kg versus 2.4±2.9 kg). No effects of the HCA were observed on appetitive variables. The active treatment group did not exhibit better dietary compliance or significant correlations between appetitive variables and energy intake or weight change. This study does not support a satiety effect of HCA. © 2000 Elsevier Science Inc. All rights reserved.

Keywords: *Garcinia cambogia*; Hydroxycitric acid; Hunger; Weight loss; Human; Appetite

1. Introduction

Dietary approaches for the management of obesity have been largely unsuccessful due, in part, to feelings of hunger that undermine adherence to weight loss regimens. Pharmacologic agents designed to suppress hunger have promoted weight loss, but are often accompanied by unacceptable side effects. Amphetamine-based anorexiants are effective in some patients, but leave them feeling anxious and are prone to abuse and chemical dependency [33]. This limits their long-term use and cessation typically results in prompt regain of body weight. Beta-phenethylamine derivatives have lower abuse potential but may still cause insomnia, anxiety and irritability [33]. While useful in many patients, their limited efficacy prompted research into a new class of agents, ones acting on serotonergic neurotransmission. Dexfenfluramine hydrocholoride and fenfluramine hydrochloride were widely effective, but were implicated in the development of cardiac valvulopathy [15] and withdrawn from the market. The most recent introduction in this class of drugs, Sibutramine, a serotonin-and norepinephrine-reuptake inhibitor, appears promising, but may increase blood pressure and heart rate in some patients [16]. It also has the potential to promote dependency if abused [16]. Attempts to manipulate satiety hormones such as cholecystokinin or bombesin to achieve sustained weight loss have proven elusive [17].

The limited success and potential complications of these pharmacologic weight loss aids has led to a large and growing market for alternative therapies such as herbal products. *Garcinia cambogia*, grown primarily in Southeast Asia, is one popular representative. The dried and cured pericarp of the fruit of this species contains up to 30% by weight of (−)-hydroxycitric acid (HCA) [19]. These rinds are used in regional cooking practices and are reported to make meals more filling [4]. This claim is bolstered by cursory observations from clinical studies [2]. A satiety effect has been demonstrated experimentally in rats and associated with weight reduction [26,36,37]. Because HCA does not appear to enter the brain, it does not elicit CNS side effects that may limit its acceptability.

HCA may promote weight reduction through suppressed de novo fatty acid synthesis, increased lipid oxidation and reduced food intake [22]. Enhanced satiety may account for the reported suppression of energy consumption. One potential mechanism accounting for the

* Corresponding author. Tel.: +1-765-494-0662; fax: +1-765-494-0674.

E-mail address: mattes@cfs.purdue.edu (R.D. Mattes).

PII: S0031-9384(00)00321-8

satiety effect of HCA may involve inhibition of ATP citrate lyase. This would limit the availability of acetyl coenzyme A (acetyle CoA) for lipid synthesis during carbohydrate feeding. As a result, carbon is diverted to glycogen synthesis. Based primarily on studies with mice [7,8] and rats [24], it has been argued that glycogen levels serve as a primary signal for energy regulation. However, this has been questioned by findings from human clinical trials [31,34]. Further, the efficiency of carbohydrate conversion to fat under conditions of energy excess in humans is extremely low [13] so inhibition of this pathway would be expected to hold limited consequence.

A second possible mechanism for an anorectic effect of HCA holds that by reducing acetyl CoA, malonyl CoA levels are depressed thereby reducing negative feedback on carnitine acyltransferase [21]. This leads to increased lipid transport into the mitochondria and inefficient oxidation with resultant ketone body formation. Ketones are purported appetite suppressants, however, several groups have failed to observe an association between ketosis and reported hunger level [3,32].

Despite an hypothesized prominent role of HCA-induced satiety on reduced energy intake and weight loss, there has been little experimental evaluation of this action in humans. Given the mechanistic issues raised above, recent evidence that HCA may not promote weight loss [14] and widespread use of products containing HCA for weight management, the question of whether HCA is an appetite suppressant warrants further consideration. The present study was designed to assess the effect of *G. cambogia* on appetitive indices and their relationship with weight loss during moderate energy restriction.

2. Methods

2.1. General protocol

Participants were recruited by public advertisement into a randomized, double-blind, placebo-controlled, parallel-group design study. During an initial baseline visit, all participants completed health, demographic and dietary restraint questionnaires, had their body weight and composition determined, completed chemosensory function tests and received dietary guidance. They were then randomly assigned to receive either caplets of *G. cambogia* or placebo. A log of hunger ratings and activities was kept over the next 24 h. During that week, participants were called twice and asked to keep 24-h diet records. One week after the initial meeting, they began their 12-week diet. Exercise was encouraged, but no formal regimen was prescribed. Diet records and hunger and activity logs were kept and chemosensory function was assessed during weeks 4, 8 and 12. At the end of weeks 2, 4, 6, 8, 10 and 12, participants reported to the laboratory for repeat assessments of body weight and composition. The protocol was approved by the Human Subjects Review Committee of Purdue University.

2.2. Subjects

Participant eligibility criteria included: 18–65 years old; 10–50 lb over ideal body weight [23]; interested in losing 10–20 lb; not adhering to any prescribed diet or taking medications (except birth control); and self-reported normal taste and smell function. A total of 167 individuals were recruited. An error in coding of pill bottles provided to the researchers (detected after the study, but prior to data analyses) resulted in 28 participants receiving a mixture of active and placebo pills. Thus, these participants were excluded from analyses. Based upon pill counts (ingestion of at least 80% of the administered caplets) and attendance at requisite evaluation sessions, a total of 106 individuals were deemed compliant with study procedures. Among the non-compliant group, 20 had been assigned to active treatment and 13 to placebo. Only 17 of the eligible sub-sample were male. Because the small number of males precluded meaningful gender-specific analyses and there are reports of sex differences in appetitive ratings [24,28,43], including to HCA treatment [2], as well as well known differences in energy intake, analyses were focused on the 89 compliant females. Eighty-seven participants were Caucasian, with one African American and one Asian. Table 1 contains other baseline characteristics of the total sample as well as the active treatment and placebo treatment groups. Only disinhibition scores differed significantly between groups ($t=2.07$, $p=0.042$).

Table 1
Participant characteristics

	Total sample ($N=89$)	Active treatment ($N=42$)	Placebo treatment ($N=47$)
Age (years)	42.7±10.0	40.97±10.4	44.0±9.5
Body mass index (kg/m^2)	28.6±0.5	28.3±0.6	28.8±0.7
Body weight (kg)	75.6−11.5	75.5−10.2	75.8−12.6
% Body fat	33.6±12.7	32.4±9.0	34.8±15.3
Weight loss goal (kg)	12.9±6.5	12.1±5.3	13.7±7.4
Cognitive restraint (Three-Factor Eating Questionnaire (TFEQ))	9.6±4.2	9.8±3.8	9.6±4.5
Disinhibition (TFEQ)	8.7±3.4	7.9±3.2	9.3±3.4
Hunger (TFEQ)	6.3±3.1	6.3±3.0	6.2±3.3

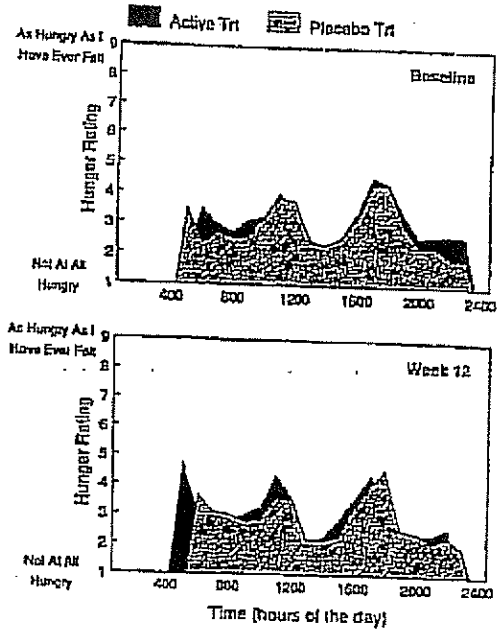


Fig. 1. Self-reported hourly hunger ratings obtained over a 24-h period on a nine-point category scale during the pre-treatment week and week 12 of treatment by participants receiving active treatment or placebo.

2.3. Treatment

Participants were counseled to adhere to a 1200-kcal exchange diet [1] that contained about 30% of energy from fat. They were provided reference materials, recipes and trained to estimate portion sizes with true-size portion charts. Active treatment participants were required to ingest two 400-mg caplets of *G. cambogia* or matched placebo three times per day (30–60 min before each meal). The source of HCA used in the study was *G. cambogia* extract (Citrin® standardized for a minimum of 50% HCA). Its purity was determined by HPLC. Thus, the total dose of HCA in the active treatment group was 1.2 g/day. Placebo treatment participants took identical caplets at the same schedule.

2.4. Appetitive questionnaires

Hunger, desire to eat, prospective consumption (how much food do you think you could eat right now?) and fullness (the primary appetitive questions) were evaluated by having participants indicate the intensity of the sensation they ascribed to each on a nine-point category scale each waking hour for 1 day at baseline and during weeks 4, 8 and 12. End anchor descriptors are listed in Figs. 1 and 2. In addition, participants indicated how intensely they experienced feelings of stomach growling, headache, thirst, irritability, itchiness and distractability (the ancillary appetitive questions) on scales ranging from "not at all" to "extremely." During another baseline day and weeks 4, 8 and 12, participants also coded hunger by outlining the place(s) on a gender-appropriate human figure where they felt the sensations they associate with hunger occurred [9] These areas were cut out of the form and weighed. They were coded into three regions — head and neck, trunk, limbs.

2.5. Restraint

Dietary restraint was assessed by the TFEQ [35].

2.6. Body weight and composition

Body weight was measured on a clinical scale with subjects wearing only a hospital gown. They voided just prior to weighing. Measurements were obtained at approximately the same time of day for each individual. Fat mass, fat-free mass and body water were determined by bioelectrical impedance analysis (Tanita Body Fat Analyzer, TBF-105, Tanita, Skokie, IL).

2.7. Dietary assessment

Energy and nutrient intake were determined with version 7.2 of The Food Processor nutrient database (ESHA Research, Salem, OR).

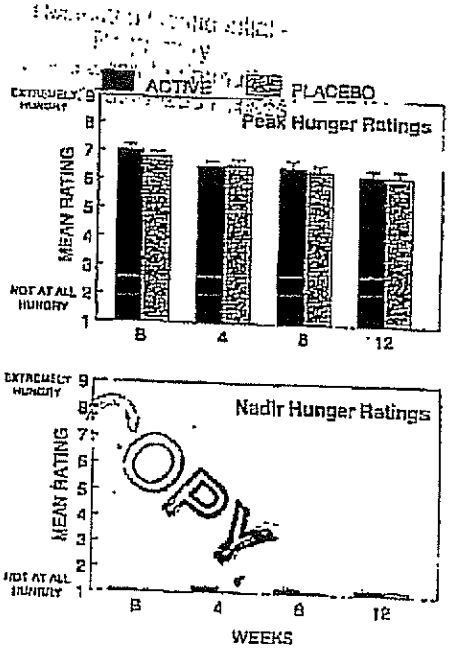


Fig. 2. Mean (±SE) peak and nadir self-reported hunger ratings obtained over a 24-h period prior to treatment (B) or at weeks 4, 8 and 12 of treatment with active compound or placebo.

2.8. Energy expenditure

Energy expended in physical activity was determined by questionnaire [30] completed at baseline and weeks 4, 8 and 12.

2.9. Sensory function

Participants rated an array of commercially available foods for sensation intensity using nine-point category scales with end anchors of "no (sweetness, saltiness, fat) at all" and "extremely (sweet, salty, high fat)." Pleasantness was also rated on a nine-point category scale with end anchors of "extremely pleasant" and "extremely unpleasant." Single bite-sized samples of foods were presented in random order and consumed. A water rinse was interspersed between samplings. Ratings were obtained at baseline and weeks 4, 8 and 12. Seventeen foods were selected to be representative of eight overlapping general categories.

Low sweet–low fat	Peaches Lite (Del Monte Foods, San Francisco, CA), Golden Loaf, fat-free and cholestrol-free (Entemanns Foods, Totowa, NJ)
High sweet–low fat	Glazed Donuts Light (Entemanns Foods), Peaches in Heavy Syrup (Del Monte Foods), Fat-Free Vanilla Ice Cream (Prairie Farms Dairy, Carlinville, IL)
Low sweet–high fat	All Butter Loaf (Entemanns Foods)
High sweet–high fat	Glazed Buttermilk Donuts (Entemanns Foods), Vanilla Ice Cream (Prairie Farms Dairy), Honey-Roasted Peanuts (Nabisco Foods, Winston-Salem, NC)
Low salt–low fat	White Corn, air-popped (American Popcorn, Sioux City, IA), Unsalted Original Sourdough Recipe Hard Pretzels (Wege Pretzel, Hanover, PA)
High salt–low fat	Original Sourdough Pretzels (Wege Pretzel), Low-Fat Original Potato Crisps (Frito-Lay, Plano, TX)
Low salt–high fat	White Corn, air-popped (American Popcorn), coated with salt-free butter (Land O' Lakes, Arden Hills, MN), Unsalted Cocktail Peanuts (Nabisco Foods)
High salt–high fat	Cocktail Peanuts (Nabisco Foods), Potato Chips (Frito-Lay)

2.10. Statistical analysis

Body weight, energy and macronutrient intake, appetitive ratings and sensory function were explored by repeated measures analysis of variance with treatment as a between group factor. Where appropriate, paired *t*-tests were used for post hoc comparisons. For the appetitive variables, the primary metric used was the mean self-reported rating during the time each individual was awake on a recording day. Associations between the appetitive variables and both dietary intake indices and weight loss outcome were assessed by Pearson correlation coefficients. The criterion for statistical significance was set at $p<0.05$, but where multiple comparisons were conducted, the Bonferroni correction was applied.

3. Results

A statistically significant loss of weight was observed over the 12-week study period in both the active ($t=7.80$, $p<0.001$) and placebo ($t=5.65$, $p<0.001$) treatment groups. The mean loss with active treatment was 5.7 ± 3.1 kg whereas the value was 2.4 ± 2.9 kg for the placebo group. The difference in weight loss between groups was also statistically significant ($t=2.26$, $p=0.026$). The decrease in fat mass was not significantly different between groups (active $=-4.1\%$ and placebo $=-3.0\%$), but the reduction in waist circumference was significant (active $=-3.96$ cm, placebo $=-2.22$ cm ($t=2.72$, $p=0.008$)). Relative to baseline, both groups reported significant reductions in energy consumption during the diet period (-1756 ± 409 kJ/day — active, -1574 ± 322 kJ/day — placebo). Mean daily intake tended to be lower during active treatment compared to placebo (5534 ± 315 versus 6191 ± 239 kJ/day), but the difference was not significant ($t=1.68$, $p<0.1$). There was no significant group difference in energy expenditure at any time point or a change over time.

Fig. 1 depicts the hunger patterns of participants at baseline and the end of the 12-week study. Because participants awoke and retired at different times of day, data are presented only when ≥ 10 participants were awake. Between 800 and 2200 h, ≥ 30 individuals were awake in each group. Ratings were coded as missing when participants were asleep. While hunger did change over the 24-h recording periods (e.g. baseline — $F(14,728)=8.44$, $p<0.001$; week 12 — $F(14,560)=8.93$, $p<0.001$), no significant group differences were observed at any time point during baseline or weeks 4, 8 or 12. Mean ratings were also comparable across the study period. Peak and nadir values were similar between the groups at baseline and at the end of weeks 4, 8 and 12 and were stable over the study period (Fig. 2). Group variance in reported hunger was significantly greater in the active treatment group at baseline (F test for variance, $p<0.05$), but the group variances during treatment were not significantly different.

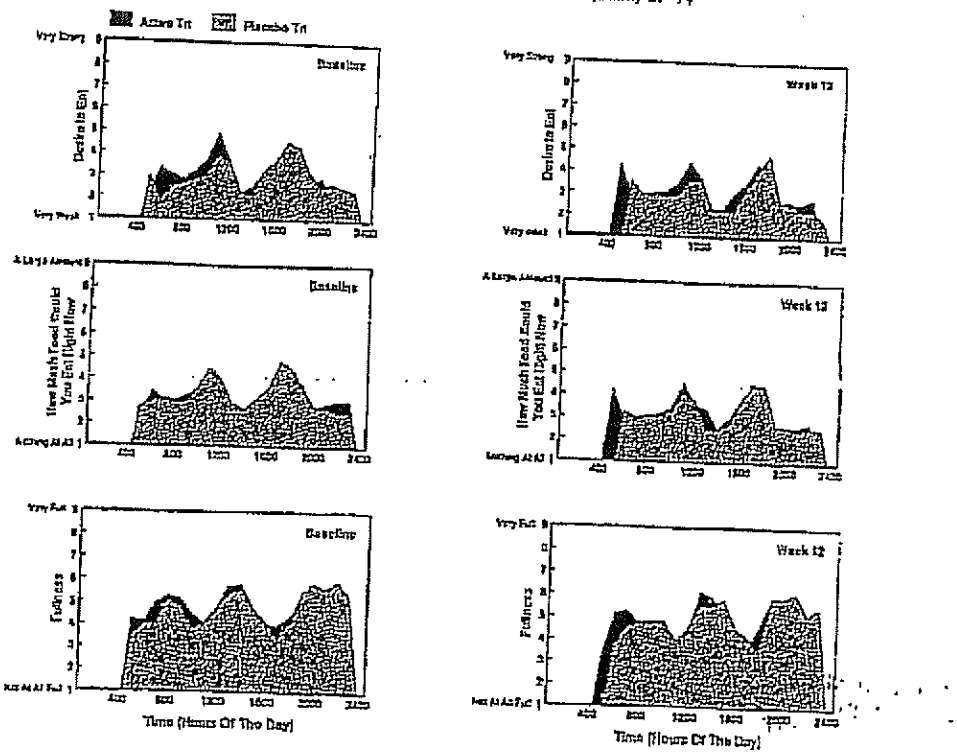


Fig. 3. Self-reported hourly "desire to eat," "prospective consumption" and "fullness" ratings obtained over a 24-h period on a nine-point category scale during the pre-treatment week and week 12 of treatment by participants receiving active treatment or placebo.

The patterns of responses for desire to eat, prospective consumption and fullness at baseline and week 12 are presented in Fig. 3. Active treatment participants reported a higher desire to eat than the placebo treated participants only at 600 h during baseline ($t=3.07$, $p=0.004$). This isolate finding is likely artifactual. Fullness and prospective consumption ratings were similar at all time points from baseline to the end of the study.

Table 2 contains self-reported 24-h mean appetite-related sensations by active and placebo treated participants at baseline and weeks 4, 8 and 12. Mean thirst ratings were higher during baseline relative to all other assessments (all $p<0.05$) in the full sample. However, the treatment effect and time by treatment interaction were not significant. No other significant treatment or time effects were observed. Ratings of itchiness, which were not expected to vary in relation to the treatment, also did not differ between groups or over time.

With a correction for multiple testing, there were no significant group differences in the rated sweetness, saltiness, fat level or pleasantness of the test foods at baseline or any time during treatment.

Baseline mean appetitive sensations did not correlate significantly with weight change in the full sample (Pearson correlation coefficients ranged from −0.19 (hunger) to 0.05 (fullness), placebo sub-group ($r=-0.24$ (hunger) to 0.12

Table 2
24-h mean (SD) appetite-related sensations (and a malingering check "itchiness" at baseline and weeks 4, 8 and 12 treatment for participants receiving active treatment and placebo. Ratings of 1.0 = not at all, 9.0 = extremely

	Baseline		Week 4		Week 8		Week 12	
	Active	Placebo	Active	Placebo	Active	Placebo	Active	Placebo
Thirst	2.9±1.4	3.3±1.6	2.9±1.6	2.8±1.6	2.5±1.2	2.8±1.4	2.7±1.4	2.7±1.5
Stomach growl	1.8±0.6	1.7±0.6	1.6±0.5	1.6±0.7	1.6±0.6	1.6±0.6	1.7±0.7	1.5±0.5
Headache	1.5±0.7	1.4±0.4	1.5±1.2	1.4±0.6	1.3±0.5	1.3±0.5	1.5±0.8	1.2±0.4
Distracted	1.4±0.7	1.5±0.9	1.5±0.6	1.6±0.8	1.3±0.7	1.6±1.2	1.3±0.8	1.5±0.9
Irritable	1.5±0.8	1.3±0.6	1.4±0.6	1.3±0.5	1.3±0.6	1.4±0.8	1.4±0.6	1.4±0.8
Itchiness	1.1±0.2	1.1±0.2	1.0±0.0	1.1±0.3	1.1±0.2	1.1±0.2	1.2±0.5	1.2±0.6

(fullness)) or active treatment sub-group ($r = -0.08$ (hunger) to 0.26 (fullness)). Among the placebo-treated participants, the only appetitive variable significantly correlated with weight change was mean hunger ratings at week 8 ($r = -0.40$, $p = 0.01$). No significant associations were observed in the active treatment group at any time point. Similarly, correlations between appetitive variables and energy intake or change of energy intake were of a low order and not statistically significant. Among active treatment participants, correlation coefficients between hunger ratings at baseline and treatment weeks 4, 8 and 12 ranged from −0.21 to 0.02 for energy intake and from −0.16 to 0.20 for change of energy intake from baseline. Correlations between the appetitive variables of desire to eat, prospective consumption and fullness and the intake variables of energy consumption and change of energy consumption ranged from −0.30 to 0.27.

4. Discussion

Consistent with the prescribed and reported reduction of energy intake, participants in both the active and placebo treated groups lost weight over the study period. The active treatment group achieved a significantly, albeit modest in absolute terms, greater reduction. This finding is consistent with several early reports [5,38], but not with a recent, larger and more vigorously controlled trial [13]. However, such comparisons must be made with caution as there were differences in the formulations and doses administered and study populations. Earlier reports were typically based on combination products (e.g., HCA plus chromium) (e.g., Ref. [2,5,10]) so efficacy cannot be ascribed to the HCA alone. Interestingly, more consistent weight loss is reported with lower doses of HCA (i.e., approximately 750 mg/day [2,5,10]) compared to higher doses (i.e., 1300–1500 mg/day [14,29,39]). Further, several had small sample sizes [5,39] and/or lacked a placebo control [2]. The work by Heymsfield et al. [14], which yielded no effect, involved males and females whereas the present report is limited to females. In fact, the males in our study exhibited more variable weight responses and if included in the sample, the significant difference from placebo treatment was eliminated. Heymsfield et al. [14] reported controlling for gender did not influence their findings, but our data suggest a gender-specific weight-loss response remains a possibility. Additionally, studies of rats suggest obese animals are more resistant to the weight reducing effects of HCA than the lean [11]. The study population used by Heymsfield et al. [14] included a higher proportion of markedly obese individuals than the present sample.

The primary focus of our work concerned the effects of HCA on appetitive variables and whether these could account for any noted effects on weight loss. The association between appetitive sensations, food intake and body weight is weak in non-dieting and dieting, free-living individuals [6,20,26,40,41] but pharmacologic enhancement of satiety has proven effective at reducing energy intake and weight [12,25]. The present data on appetitive indices are unequivocal. No significant treatment effects were observed on mean, peak or nadir hunger ratings, mean ratings of desire to eat, prospective consumption, fullness or sensations of thirst, stomach growling, headache, distraction, irritability or, as a check on malingering, itchiness. Prior support for an appetitive effect was based on anecdote [4] and data interpreted without a control treatment or pure HCA formulation [2]. The appetitive indices also were not significantly associated with energy intake or body weight change within the active treatment participants. An association between satiety effects and weight reduction has been reported in rats [27,37,38]. However, the effect is transient [11]. The association was examined at weeks 4, 8 and 12 of this study and was not apparent at any time point. It is possible that it lasted less than 4 weeks. A diminution of appetite suppression over this time frame has been noted [2] yet, interestingly, weight loss reportedly continued in that study. The weak and transient nature of appetitive effects of HCA raise questions about its clinical significance. While negative findings are always open to methodological questions, the consistency of our data across appetitive indices, larger sample size and use of more rigorous methodology lends credence to our findings. Unlike most other published work, our study also entailed ingestion of the active pills 30–60 min prior to meals when, based on animal studies, the HCA reaches peak efficacy [36]. The administered dose was modest and blood samples were not collected to confirm effective plasma levels were achieved, but the weight loss results suggest the dose was adequate to elicit physiological effects.

Increased blood ketones and hepatic or muscle glycogen levels have been posited as potential mechanisms for the satiety effect of HCA [21,22]. These indices were not measured in the present study but two recently published trials [14,18] involving participants on diets with macronutrient compositions similar that used here, have failed to note shifts associated with HCA use.

Alteration of the rewarding properties of foods can lead to reduced intake independent of hunger status [42]. However, the lack of effect of HCA on either taste intensity or hedonic ratings for foods suggests this also is unlikely to account for the present findings.

To the extent that hunger sensations are sufficiently unpleasant that they compromise dietary compliance, it was hypothesized HCA would lead to higher rates of dietary adherence relative to placebo-treated controls. However, study attrition rates were comparable in the two groups (20 from active and 13 from placebo), as noted by others [14]. These data suggest the addition of HCA does not promote improved compliance with a reduced energy diet. However, given the lack of effect on hunger, they do not address the more general question of whether amelioration of hunger serves this function.

There are several qualifications that warrant comment in this study. First, the study of appetitive properties of HCA under conditions of energy restriction could be viewed as problematic if the diet promoted extreme sensations. However, this did not occur with the mild restriction imposed as evidenced by ratings falling in the middle range of the response scales. Second, given that an energy-restricted diet would prevent the required enzyme alterations (acetyl CoA–malonyl CoA) that lead to altered substrate metabolism and satiety, the concurrent dietary restriction could have hampered induction of HCA's satiety effects. However, the prescribed diet was only mildly energy restricted and still contained at least 30% of energy from fat. Thus, it likely reflected conditions under which HCA would be used by consumers. Third, it may be that HCA is more effective at moderating weight gain [11] than promoting weight loss. This was not tested, but if true, the compound may be more useful for weight maintenance after an initial loss.

Acknowledgment

This work was funded by a grant from the SlimFast Nutrition Institute.

References

[1] American Dietetic Association. Handbook of clinical dietetics. 2nd ed. New Haven: Yale Univ. Press, 1992.
[2] Badmaev V, Majeed M. Open field, physician controlled, clinical evaluation of botanical weight loss formula Citrin™, Nutracon '95: nutriceuticals, dietary supplements and functional foods. July 11–13. Las Vegas, NV.
[3] Baird IM, Parsons RL, Howard AN. Clinical and metabolic studies of chemically defined diets in the management of obesity. Metabolism 1974;23:651–7.
[4] Clouatre D, Rosenbaum ME. The diet and health benefits of HCA (hydroxycitric acid). New Canaan, CT: Keats Publishing, 1994.
[5] Conte AA. A non-prescription alternative in weight reduction therapy. Bariatrician 1993;23:17–8.
[6] de Graaf C, Jas P, van der Knoy K, Leenen R. Circadian rhythms of appetite at different stages of a weight loss programme. Int J Obes 1993;17:521–6.
[7] Flatt J-P. McCollum Award Lecture, 1995: Diet, lifestyle, and weight maintenance. Am J Clin Nutr 1995;62:820–36.
[8] Flatt J-P. Glycogen levels and obesity. Int J Obes 1996;2:S1–S11.
[9] Friedman MI, Ulrich P, Mattes RD. A figurative measure of subjective hunger sensations. Appetite 1999;32:395–404.
[10] Gironi M, DeBernardi M, Contos S, Tripodi S, Ventura P, Cusmino C, Marletta M. Dose effect in lipid-lowering activity of a new dietary integrator (chitosan, *Garcinia cambogia* extract and chrome). Acta Toxicol Ther 1996;17:25–40.
[11] Greenwood MRC, Cleary MP, Gruen R, Blase D, Stern JS, Triscari AC, Sullivan AC. Effect of (−)-hydroxycitrate on development of obesity in the Zucker obese rat. Am J Physiol 1981; 240: 372–8.
[12] Hansen DL, Toubro S, Stock MJ, Macdonald IA, Astrup A. Thermogenic effects of sibutramine in humans. Am J Clin Nutr 1998; 68:1180–6.
[13] Hellerstein MK, Schwarz J-M, Neese RA. Regulation of hepatic de novo lipogenesis in humans. Annu Rev Nutr 1996; 16:523–57.
[14] Heymsfield SB, Allison DB, Vasselli JR, Pietrobelli A. *Garcinia cambogia* (hydroxycitric acid) as a potential antiobesity agent. JAMA 1998;280:1596–600.
[15] Khan MA, Herzog CA, St. Peter JV, Hartley GG, Madlon-Kay R, Dick CD, Asinger RW, Vessey JT. The prevalence of cardiac valvular insufficiency assessed by transthoracic echocardiography in obese patients treated with appetite-suppressant drugs. N Engl J Med 1998;339:713–8.
[16] King DJ, Devaney N. Clinical pharmacology of sibutramine hydrochloride (BTS 54 524), a new antidepressant, in healthy volunteers. Br J Pharmacol 1988;26:607–11.
[17] Kopelman PG, Reitz AB. Pharmacological treatment of obesity: therapeutic strategies. J Med Chem 1999;42:181–201.
[18] Kriketos AD, Thompson HR, Greene H, Hill JO. (−)-Hydroxycitric acid does not affect energy expenditure and substrate oxidation in adult males in a post-absorptive state. Int J Obes 1999;23:867–73.
[19] Lewis YS, Neelakantan S. (−)-Hydroxycitric acid — the principal acid in the fruits of *Garcinia cambogia*. Dear Psytochem 1965;4:619–25.
[20] Mattes RD, Hunger ratings are not a valid proxy measure of reported food intake in humans. Appetite 1990;15:103–13.
[21] McCarty M, Majeed M. The pharmacology of Citrin. In: Majeed M, Rosen R, McCarty M, Conte A, Patil D, Bunym E, editors. Citrin.® A revolutionary, herbal approach to weight management. Burlingame, CA: New Editions Publishing, 1994. pp. 34–52.
[22] McCarty MF. Promotion of hepatic lipid oxidation and gluconeogenesis as a strategy for appetite control. Med Hypotheses 1994; 42:215–25.
[23] 1983 Metropolitan height and weight tables, vol. 64. New York: Stat Bull Metrop Insur, 1983, p. 3 (January–June).
[24] Monello LF, Seltzer CC, Mayer J. Hunger and satiety sensations in men, women, boys and girls: a preliminary report. Ann NY Acad Sci 1965;131:593–602.
[25] Novin D, Robinson K, Colbreth LA, Tordoff MG. Is there a role for the liver in the control of food intake? Am J Clin Nutr 1985; 42: 1050–62.
[26] Pasquali R, Besteghi L, Castelvitri F, Melchionda N, Del'Abbo G, Zoccoli L, Barbara L, Tassoni U. Mechanisms of action of the intragastric balloon in obesity: effects on hunger and satiety. Appetite 1990; 15:3–11.
[27] Rao RN, Sakariah KK. Lipid-lowering and antiobesity effect of (−) hydroxycitric acid. Nutr Res 1988;8:209–12.
[28] Rolls BJ, Fedoroff IC, Guthrie JF, Laster LJ. Effects of temperature and mode of presentation of juice and hunger, thirst and food intake in humans. Appetite 1990;15:199–208.
[29] Rothacker DQ, Waltman BE. Effectiveness of a *Garcinia cambogia* and natural caffeine combination in weight loss: a double-blind placebo-controlled pilot study. Int J Obes 1997;21:53.
[30] Sallis JF, Haskell WL, Wood PD, Formann SP, Rogers T, Blair SN, Paffenbarger JRRS. Physical activity assessment methodology in the five-city project. Am J Epidemiol 1985;121:91–106.
[31] Shetty PS, Prentice AM, Goldberg GR, Murgatroyd PR, McKenna RJ, Stubbs RJ, Volschenk PA. Alterations in fuel selection and voluntary food intake in response to isoenergetic manipulation of glycogen stores in humans. Am J Clin Nutr 1994;60:534–43.
[32] Silverson JT, Stark JE, Buckle RM. Hunger during total starvation. Lancet 1966;1:343–4.
[33] Silverstone T. Appetite suppressants: a review. Drugs 1992;43:820–36.
[34] Stubbs RJ, Murgatroyd PR, Goldberg GR, Prentice AM. Carbohydrate balance and the regulation of day-to-day food intake in humans. Am J Clin Nutr 1993;57:897–903.
[35] Stunkard AJ, Messick S. The three-factor eating questionnaire to measure dietary restraint, disinhibition, and hunger. J Psychosom Res 1985;29:71–83.
[36] Sullivan AC, Hamilton JG, Miller ON, Wheatley VR. Inhibition of lipogenesis in rat liver by (−)-hydroxycitrate. Arch Biochem Biophys 1972;150:183–90.

ELSEVIER

NUTRITION RESEARCH

Nutrition Research 24 (2004) 45–58

www.elsevier.com/locate/nutres

Efficacy of a novel, natural extract of (–)-hydroxycitric acid (HCA-SX) and a combination of HCA-SX, niacin-bound chromium and *Gymnema sylvestre* extract in weight management in human volunteers: A pilot study

Harry G. Preuss[a,*], Debasis Bagchi[b], Manashi Bagchi[b], C.V. Sanyasi Rao[c], S. Satyanarayana[d], Dipak K. Dey[e]

[a]*Dept. of Physiology and Biophysics, Georgetown University Medical Center, Med-Dent Building, Room 103 SE, 3900 Reservoir Road NW, Washington, DC 20057, USA*

[b]*Dept. of Pharmacy Sciences, Creighton University Medical Center, 2500 California Plaza, Omaha, NE 68178, USA*

[c]*Dept of General Medicine, ASR Academy of Medical Sciences, Elluru, AP, India*

[d]*Dept of Pharmacy, Andhra University, Visakhapatnam, AP, India*

[e]*Dept. of Statistics, University of Connecticut, Storrs, CT 06269, USA*

Received 9 June 2003; received in revised form 18 September 2003; accepted 19 September 2003

Abstract

In this pilot study, the efficacy of a novel, natural extract of a highly bioavailable, calcium-potassium salt of (–)-hydroxycitric acid (HCA-SX) alone and in combination with a niacin-bound chromium (NBC) and *Gymnema sylvestra* extract (GSE) was evaluated for weight loss in moderately obese subjects by monitoring changes in body weight, body mass index (BMI), appetite, lipid profiles, serum leptin and serotonin levels, and enhanced excretion of urinary fat metabolites. *Garcinia cambogia*-derived (–)-hydroxycitric acid (HCA) has been shown to reduce appetite, inhibit fat synthesis and decrease body weight without stimulating the central nervous system. NBC has shown the ability to restore insulin function, metabolize fat, turn protein into muscle, and convert sugar into energy, which plays a role in appetite regulation and facilitates weight loss. *Gymnema sylvestre* is a traditional herb that helps to promote weight loss possibly through its ability to reduce cravings for sweets and control blood sugar levels. A randomized, double-blind, placebo-controlled human clinical study was conducted in thirty obese subjects (ages 21-50, BMI>26 kg/m^2) for eight weeks in Elluru, India. The subjects were randomly divided into three groups (10 subjects/group) and given HCA-SX 4,667 mg (60% HCA providing 2,800 mg HCA/day) (Group A), a combination of HCA-SX 4,667 mg, NBC 4 mg (providing 400 μg elemental Cr) and GSE 400 mg (providing 100 mg gymnemic acid) (Group B), or placebo (Group C) daily in 3 equally divided doses 30-60 min before each meal. This

0271-5317/04/$ – see front matter
doi:10.1016/j.nutres.2003.09.007

Kushner Attachment E

HCA-SX dose was extrapolated from previously conducted *in vitro* and *in vivo* studies. In addition, subjects received 2,000 kcal diet/day and underwent a 30 min/day supervised walking program, 5 days/week. At the end of 8 weeks, body weight and BMI decreased by 6.3%, respectively, in Group A. Food intake was reduced by 4%. Total cholesterol, LDL and triglycerides levels were reduced by 6.3%, 12.3% and 8.6%, respectively, while HDL and serotonin levels increased by 10.7% and 40%, respectively. Serum leptin levels were decreased by 36.6%, and the enhanced excretion of urinary fat metabolites, including malondialdehyde (MDA), acetaldehyde (ACT), formaldehyde (FA) and acetone (ACON), increased by 125-258%. Under these same conditions, Group B reduced body weight and BMI by 7.8% and 7.9%, respectively. Food intake was reduced by 14.1%. Total cholesterol, LDL and triglyceride levels were reduced by 9.1%, 17.9% and 18.1%, respectively, while HDL and serotonin levels increased by 20.7% and 50%, respectively. Serum leptin levels decreased by 40.5% and enhanced excretion of urinary fat metabolites increased by 146-281%. Group C reduced body weight and BMI by only 1.6% and 1.7%, respectively, food intake was increased by 2.8%, and LDL, triglycerides and total cholesterol decreased by 0.8%, 0.2% and 0.8%, respectively. HDL were reduced by 4.1% while serum leptin levels were increased by 0.3%, and excretion of urinary fat metabolites did not change in MDA, ACT and FA, and marginally increased in the case of ACON. No adverse effects were observed. Results demonstrate that HCA-SX and, to a greater degree, the combination of HCA-SX, NBC and GSE can serve as safe weight management supplements.

Keywords: Obesity; Lipids; Urinary metabolites; *Garcinia cambogia*; Niacin-bound chromium; *Gymnema sylvestre*

1. Introduction

Current statistics demonstrate that more than half of U.S. adults are overweight (61%), defined as having a body mass index (BMI) greater than 25 kg/m^2, while more than a quarter (26%) of U.S. adults are obese, having a BMI of greater than 30 kg/m^2 [1,2]. Sixty three percent of men and 55% of women are now overweight or obese in this country [3]. Providing an even worse outlook, national data indicate that 10.5-15.5% of children ages 6 through 19 are severely overweight [4]. According to the World Health Organization, there are over 300 million obese adults globally [5]. Low levels of physical activity and sedentary lifestyles have generally been implicated in the worldwide trend of weight gain [6].

Obesity, resulting from an imbalance between energy intake and expenditure, is the second leading cause of premature death in America. Other potential risks of obesity include cardiovascular diseases, diabetes, cancer and hormonal imbalances in women, leading to sterility [7]. Low caloric diets with and without exercise can help with temporary weight loss. Weight loss drugs that suppress appetite, reduce food intake, increase energy expenditure and/or affect nutrient partitioning or metabolism have potential efficacy but are unfortunately frequently accompanied by adverse side effects [8]. Therefore, supplementation with safe and natural products in addition to a healthy diet and exercise may be helpful.

* Corresponding author. Tel.: + 1-202-680-1441; fax: + 1-202-687-8788.
E-mail address: preusshg@georgetown.edu (H.G. Preuss).

(–)-Hydroxycitric acid (HCA) has been reported to cause weight loss in humans without stimulating the central nervous system [9]. HCA is derived from the fruit rinds of *Garcinia cambogia*, which exhibits a distinctive sour taste and has been used for culinary purposes in Southern Asia for centuries to make meals more "filling", and has been reported to reduce food intake in experimental animals, suggesting its role in the treatment of obesity [10–14]. HCA is a competitive inhibitor of ATP-citrate lyase, an extra-mitochondrial enzyme involved in the initial steps of *de novo* lipogenesis [7,10–14]. Consequently, HCA reduces the transformation of citrate into acetyl coenzyme A, a step necessary for the formation of fatty acids in the liver. In addition, there is increased production of hepatic glycogen in the presence of HCA, which may activate glucoreceptors leading to a sensation of fullness and reduced appetite [12,15]. Earlier successful animal trials [13,14] suggest that the human dose of HCA typically recommended in dietary supplements and used in previous clinical trials (1,500 mg HCA/day) is sub-optimal. Several publications have reported the efficacy of HCA in weight management [16–20].

Acute oral, acute dermal, primary dermal, and primary eye irritation studies demonstrated the safety of HCA-SX [21]. The LD_{50} in rats was found to be greater than 5 gm/kg. HCA-SX bioavailability was found to be significantly higher in fasting individuals when consumed at least 30-60 min prior to food consumption [22].

Chromium is important for energy production and plays a role in regulating appetite. Administration of 600 μg elemental chromium as NBC (ChromeMate) in two divided doses daily over a period of 2 months to African-American women with a moderate diet and exercise regimen influenced weight and fat loss and sparing of muscle and body composition [23]. Their blood chemistries revealed no significant adverse effects [23]. Another study at the University of Texas found that young obese women consuming 400 μg of elemental chromium as NBC per day with exercise experienced significant weight loss over an eight week period. Insulin response to an oral glucose load was also lowered in the obese subjects, and no adverse effects were observed [24]. Preuss *et al.* [25] conducted a long term study for 12 months in Fischer F344/BN rats using a chronic dose of 400 μg of elemental chromium per day and no adverse effects were observed in body and organ weights, and blood chemistries [25].

Gymnema helps to promote weight control by its ability to reduce the cravings for sweets and control blood sugar levels [26,27]. A peptide isolated from Gymnema, gurmarin, has also been shown to block the sweet taste of glucose and sucrose in animal models [27]. Gurmarin temporarily binds to the sweet and bitter receptors on the tongue, thereby blocking the taste sensation and reducing sweet cravings [27]. Preuss *et al.* [28] showed a significant lowering of cholesterol with *Gymnema sylvestre* ingestion in hypertensive rats fed a high sucrose diet, whereas the placebo group showed a significant increase in cholesterol levels. *Gymnema* is regarded as very safe and has been administered (400 mg/day) to patients with insulin-dependent diabetic mellitus (IDDM) for 10-12 months with no adverse side effects [29].

The effective dose of HCA-SX was determined by a previous *ex vivo* study on serotonin release from isolated rat brain cortex [21,30] and in *in vivo* studies [13,14]. Based on these studies the human equivalency dose of HCA-SX used in the present study was calculated to be 2,800 mg/day, which is significantly greater than the 1,500 mg/day that is normally recommended in dietary supplements [16].

The present study was designed to examine the efficacy of optimal doses of HCA-SX alone and in combination with NBC and GSE given on an empty stomach in thirty human volunteers. Effects of these supplements were investigated on body weight, BMI (an indicator of obesity health risk), appetite (as determined by weighing the remaining food), lipid profiles, serum leptin levels (a biomarker of obesity regulatory gene), serotonin levels, and excretion of urinary fat metabolites (a biomarker of fat oxidation).

2. Subjects and methods

2.1. Subjects

In this study conducted in Elluru, India, each subject was obese, ages 21-50 years, with a body mass index (BMI) ranging from 30.0 to 50.8 kg/m^2 (BMI requirement was greater than 26>kg/m^2). Additional inclusion criteria consisted of having a negative pregnancy test, possessing the ability to understand the risks/benefits of the protocol, willingness to participate in a 30 min supervised walking-exercise program (5 days a week), eat the vegetarian or non-vegetarian prescribed diets of approximately 2,000 kcal/day (17% protein, 25% fat, and 58% carbohydrate) divided into three meals, sign an informed consent form, complete a standard health questionnaire, and participate in 3 clinic visits at 0, 4, and 8 weeks. Subjects were excluded if they were pregnant or nursing, presently taking other weight loss medications, had a history of thyroid disease, cardiovascular disease, or diabetes, suffered from intractable obesity, had defined weight limits or had experienced any recent, unexplained weight loss or gain. Subjects were required to fast overnight, and blood and urine samples were obtained at each clinic visit in the early morning to avoid diurinal variation. An individual diary was maintained for each subject.

Advertisements were placed in local newspapers and overweight subjects who responded and met the inclusion criteria during a screening were scheduled for a baseline visit. The evaluation included a questionnaire, physical examination, electrocardiogram, and screening blood studies. Subjects were then randomized into three groups (10 subjects/group) with equal probability through a random number generator. An Institutional Review Board approval IRB #01-001 was obtained from ASR Academy of Medicinal Sciences for this study. All subjects gave written consent prior to participation.

2.2. Weight reduction protocol

A detailed evaluation was performed at the beginning, week four, and week eight of treatment. Bodyweight, BMI, appetite, lipid profile, serum leptin and serotonin levels and excretion of urinary fat metabolites were evaluated. The patients' diaries were checked on a daily basis.

Body weights of the subjects were measured using an Essae Digi (Model DS-410) digital weighing scale (Essae-Teraoka Pvt. Ltd., Bangalore, India). Height was measured using a Benson Track and Field height scale. BMI was calculated by body weight in kilograms divided by square of height in meters. Appetite reduction was estimated by weighing the

remaining food after each meal. Lipid profile, including high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL)W.S.William Seroy*via* HPLC in conjunction with GCMS using a selective ion monitoring technique as described by Shara et al. [31].

The following six key parameters were monitored in a randomized, double-blind, placebo-controlled study over a period of eight weeks: [1] To assess whether optimal doses of HCA-SX, and the combination of HCA-SX, NBC plus GSE (HCA-SX Formula) produces a greater reduction in body weight than placebo, [2] To evaluate whether HCA-SX and HCA-SX Formula produces a greater reduction in BMI than placebo, [3] To assess whether HCA-SX and HCA-SX Formula has an inhibitory effect on appetite as compared to placebo, [4] To assess whether HCA-SX and HCA-SX Formula produces a beneficial effect on lipid profile, including LDL, HDL, triglycerides, VLDL, and total cholesterol, as compared to placebo, [5] To assess whether HCA-SX and HCA-SX Formula has an inhibitory effect on serum leptin and serotonin levels compared to placebo, and [6] To evaluate whether HCA-SX and HCA-SX Formula causes fat oxidation as estimated by enhanced excretion of urinary fat metabolites, including malondialdehyde, acetaldehyde, formaldehyde, and acetone as compared to placebo.

Subjects were divided into three groups. Group A was given a daily dose of HCA-SX 4,667 mg (60% HCA providing 2,800 mg HCA per day), Group B was given a daily dose of a combination of HCA 4,667 mg, NBC 4 mg (400 μg elemental chromium) plus GSE 400 mg (100 mg gymnemic acid), and Group C was given a placebo (microcrystalline cellulose) in three equally divided doses 30-60 min before breakfast, lunch and dinner for eight weeks.

2.3. Study materials

A natural, highly bioavailable, water-soluble, tasteless and odorless calcium-potassium salt of 60% HCA extract from *Garcinia cambogia* commercially known as Super CitriMax (HCA-SX), niacin-bound chromium supplement commercially known as ChromeMate (containing 10% elemental chromium) and a standardized extract of *Gymnema sylvestre* extract commercially known as Gymnema (GSE, GYM-250) (containing 25% gymnemic acid) were obtained from InterHealth Nutraceuticals, Inc., (Benicia, CA). Unless stated otherwise, all other chemicals and reagents were obtained from Sigma Chemical Co. (St. Louis, MO) and were of analytical grade or the highest grade available.

2.4. Dose determination

Earlier animal studies by Sullivan et al [13,14] indicate that higher levels of HCA than those typically recommended in dietary supplements for human beings are required to produce consistent and reliable results. Levels of HCA-SX used in our study were determined by extrapolation of optional micromolar concentrations of HCA required to evoke peak levels of serotonin release in rat brain cortex study [21,30]. Considering a 5-fold faster metabolism in rats compared to humans, the *ex viso* dose extrapolates to a human dose of 2,800 mg of HCA per day, which approximates the human equivalent does used in the earlier animal studies conducted by Sullivan et al. [13,14].

Table 1
Effects of placebo (Group C), HCA-SX alone (Group A) and HCA-SX formula (Group B) on body weight, BMI, and serum leptin levels in human subjects.

Group	Time Point	Body Weight (kg)	BMI (kg/m^2)	Serum Leptin (ng/ml)	Serotonin (ng/ml)
Placebo (Group C)	I	87.39 ± 5.04^a	34.0 ± 1.42^a	34.60 ± 2.58^a	220.0 ± 17.09^a
	M	86.56 ± 5.00^a	33.7 ± 1.40^a	34.60 ± 2.58^a	239.0 ± 14.16^a
	F	86.00 ± 4.95^a	33.5 ± 1.34^a	34.70 ± 2.34^a	266.3 ± 15.31^b
HCA-SX (Group A)	I	88.50 ± 6.89^a	33.6 ± 1.97^a	45.40 ± 3.54^a	216.0 ± 23.55^b
	M	88.70 ± 6.70^b	32.6 ± 1.94^a	37.30 ± 3.90^b	265.0 ± 24.85^b
	F	83.00 ± 6.80^b	31.5 ± 1.99^b	28.80 ± 3.44^c	302.0 ± 26.74^c
HCA-SX Formula (Group B)	I	87.60 ± 5.12^a	34.1 ± 1.42^a	33.80 ± 3.39^a	243.0 ± 20.92^a
	M	84.00 ± 5.03^b	32.7 ± 1.46^b	27.20 ± 3.05^b	298.0 ± 21.05^b
	F	80.80 ± 4.93^c	31.4 ± 1.39^c	20.10 ± 2.50^c	365.0 ± 22.75^c

Data are presented as group mean ± SEM. Subjects were given placebo (Group C), HCA-SX alone (Group A) or HCA-SX formula (Group B) for 8 weeks. See Subjects and methods section for details. Values with non-identical superscripts are significantly different ($p < 0.05$).

2.5. Data analysis

Two-tailed Student's t test, with a level of 5% significance, was performed on all three groups for each variable to detect any significant changes. The data set that was analyzed had eleven variables of interest, which are body weight, body mass index (BMI), low density lipoproteins (LDL), high density lipoproteins (HDL), triglycerides, very low density lipoproteins (VLDL), total cholesterol, serum leptin, serotonin, enhanced excretion of urinary fat metabolites, and remaining food. [32–34].

In each group, longitudinal data was collected for 3 time points denoted by Initial (I), Middle (M) and Final (F) for the first 10 variables, and at 8 time points for the last variable, which is "remaining food".

To compare the differences at a 5% level of significance, we have differences for "I & M", "M & F" and "I & F" for the first 10 variables. For remaining food, since data was collected at 8 time points, there are 28 possible paired differences. Basic summary statistics and test for differences with respect to least square means, among the time points was conducted for each of the variables for each group at each respective timepoint. $P<0.05$ was considered statistically significant.

3. Results

The present clinical study on HCA reported that subjects taking higher, more optimal doses of a highly bioavailable form of HCA (HCA-SX) not only had a significant weight loss, but reduced food intake, increased fat oxidation, decreased LDL, triglycerides and total cholesterol, increased HDL levels, and decreased BMI compared to placebo. The study also demonstrated some surprising new results: high doses of HCA-SX significantly lowered serum leptin levels and increased serotonin levels as determined by our previous *in vitro* and

Table 2
Effects of placebo (Group C), HCA-SX alone (Group A) and HCA-SX formula (Group B) on appetite in human subjects. Remaining food in grams on the plate as an index of appetite suppression.

Group	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Placebo (Group C)	82.22 ± 35.22^{a}	78.33 ± 27.91^{a}	101.11 ± 35.92^{a}	122.22 ± 57.80^{a}	80.00 ± 32.49^{a}	61.11 ± 31.20^{a}	22.22 ± 16.90^{b}	38.89 ± 23.24^{a}
HCA-SX (Group A)	236.50 ± 51.87^{a}	258.00 ± 80.85^{a}	237.50 ± 40.46^{a}	185.50 ± 40.56^{a}	279.00 ± 84.61^{a}	226.50 ± 56.05^{a}	342.00 ± 60.34^{b}	317.50 ± 24.42^{b}
HCA-SX Formula (Group B)	218.50 ± 26.44^{a}	216.00 ± 44.36^{a}	252.50 ± 38.69^{a}	372.50 ± 57.46^{b}	344.00 ± 79.20^{b}	418.00 ± 81.37^{b}	388.00 ± 49.12^{b}	505.00 ± 45.89^{b}

Data are presented as group mean ± SEM. Subjects were given placebo (Group C), HCA-SX alone (Group A) or HCA-SX formula (Group B) for 8 weeks. See Subjects and methods section for details. Values with non-identical superscripts in each row are significantly different ($p < 0.05$).

Table 3
Effects of placebo (Group C), HCA-SX alone (Group A) and HCA-SX formula (Group B) on lipid profile in human subjects.

Group	Time Point	LDL (mg/dL)	HDL (mg/dL)	Triglycerides (mg/dL)	VLDL (mg/dL)	Total Cholesterol (mg/dL)
Placebo	I	126.00 ± 8.70[a]	30.00 ± 2.22[a]	140.00 ± 27.99[a]	31.00 ± 5.05[a]	186.60 ± 10.98[a]
(Group C)	M	124.00 ± 7.99[a]	30.00 ± 1.62[a]	138.00 ± 27.54[a]	29.00 ± 5.88[a]	182.90 ± 10.52[b]
	F	125.00 ± 8.00[a]	29.00 ± 1.63[a]	140.00 ± 26.46[a]	31.00 ± 5.86[a]	185.10 ± 10.07[a]
HCA-SX	I	106.00 ± 5.75[a]	30.70 ± 2.48[a]	153.50 ± 31.90[a]	33.00 ± 6.60[a]	169.50 ± 7.77[a]
(Group A)	M	102.00 ± 4.67[a]	30.80 ± 2.09[a]	147.20 ± 31.42[a]	32.00 ± 6.37[a]	164.80 ± 7.21[a]
	F	93.00 ± 5.15[b]	34.00 ± 2.23[b]	140.30 ± 31.32[b]	32.00 ± 5.91[a]	158.80 ± 6.17[b]
HCA-SX	F	112.00 ± 8.12[a]	29.00 ± 1.57[a]	98.00 ± 11.27[a]	26.00 ± 2.63[a]	166.30 ± 8.56[a]
Formula	M	103.00 ± 7.54[b]	31.00 ± 1.54[b]	84.00 ± 8.43[b]	26.00 ± 2.52[a]	160.20 ± 8.62[a]
(Group B)	F	92.00 ± 7.13[c]	34.00 ± 1.75[c]	81.00 ± 9.12[c]	25.00 ± 2.83[a]	151.10 ± 7.73[b]

Data presented as group mean ± SEM. Subjects were given placebo (Group C). HCA-SX alone (Group A) or HCA-SX formula (Group B) for 8 weeks. See Subjects and methods section for details. Values with non-identical superscripts are significantly different ($p < 0.05$).

in vivo animal studies [20,29,12,13]. The addition of NBC and GSE generally caused greater significant changes in all parameters measured.

Table 1 demonstrates the changes in body weight, BMI, serum leptin and serotonin following supplementation of placebo (Group C), HCA-SX (Group A) and HCA-SX Formula (Group B) over the period of eight weeks. There was a distinct change observed at the end of four weeks and eight weeks in both Group A and Group B. In Group C, approximately 0.83 and 1.39 kg reduction in body weights were observed at the end of four and eight weeks, respectively. Under the same conditions, approximately 2.8 and 5.5 kg reduction in body weights were observed in Group A, and 3.6 and 6.8 kg reduction in body weights were observed in the Group B, respectively, at the end of four and eight weeks. Thus, at the end of eight weeks, Group C only showed a reduction of 1.7% BMI while there were a 6.3% and 7.9% reduction in BMI observed in Group A and Group B, respectively.

Group C showed no change in serum leptin levels at the end of four weeks and a 0.3% increase in serum leptin levels at the end of eight weeks, while both Group A and Group B exhibited a significant reduction. Approximately 17.8% and 36.6% reduction in serum leptin levels were observed in Group A and 19.5% and 40.5% reduction in serum leptin levels were observed in Group B, respectively, at the end of four and eight weeks. In Group C, an increase of approximately 8.6% and 21% was observed in serotonin levels at the end of four and eight weeks, respectively. However, Group A demonstrated a 23% and 40% increase and Group B demonstrated a 23% and 50% increase at the end of four and eight weeks, respectively.

Table 2 demonstrates the amount of remaining food over the period of eight weeks for each group, which reflects a trend of appetite suppression in both Group A and Group B. Group C exhibited a slight increase in food consumption of 2.8%. Approximately a 4% and 14.1% reduction in appetite was observed in Group A and Group B at the end of eight weeks, respectively.

Table 3 demonstrates the changes in lipid profiles, including LDL, HDL, triglycerides,

Table 4
Effects of placebo (Group C), HCA-SX alone (Group A) and HCA-SX formula (Group B) on enhanced excretion of urinary fat metabolites (nmoles/ml of urine)

Group	Time Point	MDA	ACT	FA	ACON
Placebo	I	0.168 ± 0.042^{a}	1.527 ± 0.345^{a}	5.034 ± 0.970^{a}	16.36 ± 1.391^{a}
(Group C)	M	0.121 ± 0.020^{a}	1.056 ± 0.178^{a}	5.423 ± 1.093^{a}	13.65 ± 2.556^{a}
	F	0.133 ± 0.026^{a}	1.338 ± 0.092^{a}	4.670 ± 0.572^{a}	18.46 ± 2.462^{a}
HCA-SX	I	0.110 ± 0.029^{a}	1.151 ± 0.131^{a}	4.380 ± 1.595^{a}	20.03 ± 1.281^{a}
(Group A)	M	0.176 ± 0.062^{a}	1.262 ± 0.159^{b}	5.981 ± 0.344^{a}	21.55 ± 1.784^{a}
	F	0.284 ± 0.077^{b}	1.672 ± 0.129^{c}	7.042 ± 0.878^{b}	25.05 ± 1.934^{b}
HCA-SX	I	0.109 ± 0.012^{a}	1.167 ± 0.088^{a}	4.011 ± 0.0375^{a}	18.65 ± 1.526^{a}
Formula	M	0.211 ± 0.072^{b}	1.387 ± 0.11^{b}	5.756 ± 0.519^{b}	24.37 ± 2.292^{b}
(Group B)	F	0.306 ± 0.081^{c}	1.720 ± 0.105^{a}	7.737 ± 0.464^{c}	27.14 ± 2.525^{c}

Data are presented as group mean ± SEM. Subjects were given placebo (Group C), HCA-SX alone (Group A) or HCA-SX formula (Group B) for 8 weeks. See Subjects and methods section for details. Values with non-identical superscripts are significantly different ($p < 0.05$).

VLDL and total cholesterol in Groups A, B and C. There was some reduction in LDL and triglycerides in Group A, however, the changes that were observed in Group B were even more pronounced. Group B demonstrated a boost in HDL levels, while little effect was observed in Group A. The overall total cholesterol level decreased significantly in both Groups A and B. Approximately 3.8% and 12.3% reduction in LDL levels were observed in Group A, while under these same conditions approximately 8% and 17.9% reduction in LDL were observed in Group B at the end of four and eight weeks, respectively. However, Group C only showed a 1.6% and 0.8% decrease in LDL levels at the end of four and eight weeks, respectively. In Group A, approximately 0.3% and 10.7% increases in HDL levels were observed and 6.9% and 20.7% increases in HDL levels were observed in Group B, respectively, at the end of four and eight weeks. No significant changes were observed in Group C. Approximately 4.1% and 8.6% reduction in triglyceride levels were observed in Group A and 15.1% and 18.1% reduction in triglyceride levels were observed in Group B, respectively, at the end of four and eight weeks, respectively. No significant changes were observed in Group C. No significant changes were observed in the VLDL levels in any of the groups. Approximately, 2.8% and 6.3% reduction in total cholesterol levels were observed in Group A and 3.7% and 9.1% reduction in total cholesterol were observed in Group B at the end of four and eight weeks, respectively. No significant changes were observed in Group C.

Table 4 demonstrates the enhanced excretion of urinary fat metabolites, including MDA, ACT, FA and ACON, which were quantified as biomarkers of fat oxidation. Approximately 125-258% increases in total urinary fat metabolites in Group A and 146-281% increases in total urinary fat metabolites in Group B were observed at the end of eight weeks, respectively, as compared to the control samples. In Group C, excretion of urinary fat metabolites did not change in MDA, ACT and FA, and marginally increased in the case of ACON at the end of eight weeks.

In Group A, MDA, ACT, FA and ACON increased by 160%, 110%, 140% and 110% at the end of four weeks, respectively, and 260%, 150%, 160% and 130% at the end of 8 weeks,

respectively. In Group B, MDA, ACT, FA and ACON increased by 190%, 120%, 140% and 130% at the end of four weeks, respectively, and 280%, 150%, 190% and 150% at the end of eight weeks, respectively. In Group C, excretion of MDA, ACT and FA decreased at the end of four eight weeks of treatment, while excretion of ACON decreased at the end of four weeks and increased at the end of eight weeks (Table 4).

Thus, both HCA-SX alone and in combination with NBC and GSE demonstrated significant reduction in body weight, BMI, LDL, triglycerides, total cholesterol and serum leptin as well as significant increases in HDL, serotonin levels and excretion of urinary fat metabolites.

3.1. Adverse events and drop outs

Twenty-nine of the initial thirty subjects completed the study. During the eight week study, adverse events were noted on a daily basis on each subject. There were no serious adverse events noted in any of the groups in this study. It is important to emphasis that the number of patients reporting adverse events in the supplemented groups was not significantly different from Group C.

One subject dropped out of this study on the 21st day for personal reasons, which was not a result of an adverse event caused by the treatment. No patient was removed or dropped out of the study as a result of an adverse event caused by the treatment.

4. Discussion

Obesity, a chronic disequilibrium between food consumption and energy expenditure, continues to be a major health problem in developed and developing countries. Obesity and its related metabolic and cardiovascular complications continue to present an escalating challenge to contemporary medicine [1–3].

HCA, found in citrus fruits such as oranges and lemons, is an organic acid similar to citric acid but its properties are remarkably different from citric acid. HCA has been shown to reduce appetite, inhibit fat synthesis, and decrease body weight without stimulating the central nervous system [11,15,21,30]. Furthermore, HCA does not cause nervousness, rapid heart rate, high blood pressure, or insomnia, symptoms that are often associated with dietary stimulants such as ephedra (Ma-Huang), caffeine or phenylpropanolamine [35]. Thus, HCA may prove to be a safe alternative to these popular diet aids [35].

In our previous studies [20,29] and in the present study, we have demonstrated other important mechanisms including the appetite suppression by HCA and serotonin release by rat brain cortex *in vitro*, regulatory roles on the lowering of lipid profiles, and increased fat oxidation as demonstrated by enhanced excretion of urinary fat metabolites.

Studies by Ramos et al. (1995) demonstrated that 500 mg HCA (CitriMax) taken three times per day before meals for 8 weeks resulted in 215% greater weight loss in twenty overweight adults than those taking a placebo. No adverse events were reported [16]. Of added importance, a significant reduction was also observed in cholesterol and triglyceride levels. Mattes and Borman (2000) demonstrated that a daily dose of 1.2 gm HCA along with

a daily diet of 5,020 kJ (1,200 kcal) for 12 weeks resulted in a significant difference in weight loss (3.7 ±3.1 vs 2.4 ±2.9 kg) compared to placebo [17]. A 6-week randomized, placebo-controlled, single-blind, cross-over study was conducted by Westerterp-Plantenga and Kovacs (2002) in twelve males and twelve females using a daily dose of 900 mg HCA for two weeks. Results demonstrated that HCA supplementation reduced 24 hr energy intake in humans, while satiety was sustained [19].

In examining the "negative studies", Heymsfeld, et al. (1998) provided what became an accepted daily dose of 1.5 gm HCA along with a diet of 5,020 kJ/day or 1,200 kCal/day diet for 12 weeks, and reported that no significant difference in weight loss was observed between the placebo and treatment groups [36]. However, several problems with the study may be responsible for the negative results. Heymsfeld, et al. (1998) quantified the HCA content, but did not assess the bioavailability of the HCA sample used in their study [36]. Many HCA products are less than 50% soluble in water and poorly absorbed. Also, the low-calorie diet (5,020 kJ/day or 1,200 kcal/day) may have accounted for the substantial decreases in body weight of both treatment and placebo groups blunting the ability of HCA to show curbed appetite and reduced food intake.

Our present study used a highly bioavailable calcium-potassium salt of HCA and the optimal dose was determined based on the mechanistic observation of serotonin release in rat brain cortex. Supplements were given on an empty stomach, since we had previously demonstrated that HCA-SX should be consumed at least 30-60 min before meals to enhance bioavailability. This more efficacious dose was extrapolated from *ex vivo* and *in vivo* studies.

Furthermore, our study was designed to better determine the effects of HCA on satiety. A 2,000 kcal/day or 8,372 kj/day was administered, and all unconsumed food was weighed as an approximation of appetite reduction. Supplementation with HCA-SX, and to a greater degree HCA-SX combined with NBC and GSE, significantly reduced appetite as determined by increased amounts of remaining food.

Perceived small weight loss in both the HCA-SX (Group A) and HCA-SX formula (Group B) group is supported by the improvement in BMI which means sparing lean muscle and burning fat, as demonstrated by enhanced excretion of urinary fat metabolites.

Another possible mechanism of action may be HCA's ability to down-regulate the gene that influences obesity and body weight. Leptin is a 167 amino acid protein hormone encoded by the obesity regulatory gene. Synthesized and secreted by adipocytes (fat cells), leptin is present in the bloodstream in amounts related to the amount of fat in the body. Leptin acts primarily on the brain, where it binds to receptors and activates signals that inhibit food intake and increase energy expenditure [37,38]. When receptor-binding activity is diminished, "leptin resistance" develops, i.e., plasma leptin levels increase and lose their ability to inhibit food intake and increase energy expenditure. Studies show that plasma leptin levels are higher in overweight than in non-overweight individuals, and higher in women than in men. Leptin has been shown to be able to modulate insulin secretion and action through these receptors [38,39]. Our findings in conjunction with earlier studies demonstrate that intracellular energy production is important for acute leptin secretion and that potassium and calcium flux may play roles in coupling intracellular energy production to leptin secretion [39,40]. We hypothesize that the calcium-potassium salt of HCA (HCA-SX) may play a role in down-regulating leptin, the obesity regulatory gene.

Serotonin (5-HT) has been implicated in the control of eating behavior and body weight [41,42]. Our previous study demonstrated HCA-SX's ability to increase the availability of serotonin in isolated rat brain cortex and serve as a mild serotonin receptor reuptake inhibitor [20,29]. It is important to note that obese subjects generally show a low level of circulating serotonin level, Our present study, demonstrated increased levels of circulating serotonin in both supplemented groups as compared to the control subjects.

The current study also suggests that HCA has the ability to augment fat breakdown. This is based upon the enhanced excretion of urinary fat metabolites following usage, including malondialdehyde (MDA), acetaldehyde (ACT), formaldehyde (FA), and acetone (ACON), markers of oxidative fat degradation. The sources of these four lipid metabolites are not entirely clear, but the increases in these products are probably due to enhanced β-oxidation of fat. Dhanakoti and Draper [43] demonstrated that urinary MDA excretion was enhanced following enhanced oxidative stress. Furthermore, the fate of radiolabeled MDA administered to rats was found to be extensively metabolized to acetate and carbon dioxide. Based on these observations, the urinary ACT identified in this study may arise from the breakdown of MDA, which is formed as a result of fat oxidation/lipid peroxidation. The enhanced formation of ACON in response to a consequence of enhanced β-oxidation is also well known [44]. Winters et al. [45,46] reported that rat liver microsomes metabolized glycerol to FA. Glycerol is a product of the metabolism of triglycerides by adipose tissue and other tissues that possess the enzyme that activates glycerol, namely, glycerol kinase. Liver and brown tissues are known to have high glycerol kinase levels [45,46]. Other possible sources of FA might include the breakdown of MDA to acetate or ACT and a one carbon fragment [43], and/or the cleavage of a one carbon fragment from acetoacetic acid with the formation of ACON. Under this situation, it should be pointed out that the triglyceride levels were significantly reduced in both HCA-SX and HCA-SX formula groups, accompanied by significant increases in urinary excretion of FA. These results suggest that these supplements may induce enhanced production of glycerol kinase in the biological system, however, this has yet to be proven.

High levels of total cholesterol, LDL cholesterol and triglycerides, as well as low levels of HDL cholesterol, are all risk factors for cardiovascular diseases, diabetes and stroke. The current study shows that supplementation with HCA-SX, and to a greater degree HCA-SX, NBC plus GSE, significantly improves blood lipid profiles.

Taken together, these studies demonstrate that optimal doses of HCA-SX alone and in combination with NBC plus GSE given on an empty stomach are safe, bioavailable, and highly efficacious diet aids that can help reduce excess, or maintain healthy body weight and BMI, and promote healthy blood lipid levels. The reduced serum leptin levels, decreased appetite, reduced food intake, and increased fat oxidation may be, at least in part, responsible for this positive outcome and decrease the risk factors for obesity related degenerative diseases and mortality.

References

[1] Jequier E. Pathways to obesity. Int J Obes Relat Metab Disord 2002;260:S12–7.

[2] Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: International survey. Br Med J 2000;320:1240–3.
[3] Roberts SB, McCrory MA, Saltzman E. The influence of dietary composition on energy intake and body weight. J Am Coll Nutr 2002;21:140S–5S.
[4] Ogden CL, Flegal KM, Carroll MD, Johnson CL. Prevalence and trends in overweight among US children and adolescents, 1999-2000. JAMA 2002;288:1728–32.
[5] World Health Organization. Report: Controlling the global obesity epidemic. Available at www.who.int/nut/obs.htm. Updated August 15, 2003.
[6] Popkin BM, Paeratakul S, Zhai F, Keyou G. A review of dietary and environmental correlates of obesity with emphasis on developing countries. Obes Res 1995;3:145S–53S.
[7] Sullivan AC, Triscari J, Cheng L. Appetite regulation by drugs and endogenous substances. Curr Concep Nutr 1983;12:139–67.
[8] Haller CA, Benowitz NL. Adverse cardiovascular and central nervous system events associated with dietary supplements containing ephedra alkaloids. N Engl J Med 2000;343:1833–8.
[9] Clouatre, D, Rosenbaum, M. The diet and health benefits of HCA. A Keats Good Health Guide. pp. 9, 1994.
[10] Sergio W. A natural food, the malabar tamarind, may be effective in the treatment of obesity. Med Hypotheses 1988;27:39–40.
[11] Jena BS, Jayaprakasha GK, Singh RP, Sakariah KK. Chemistry and biochemistry of (–)-hydroxycitric acid from *Garcinia*. J Agric Food Chem 2002;50:10–22.
[12] Lowenstein JM. Effect of (–)-hydroxycitrate on fatty acid synthesis by rat liver *in vivo*. J Biol Chem 1971;246:629–32.
[13] Sullivan AC, Triscari J, Hamilton JG, Miller ON, Wheatley VR. Effect of (–)-hydroxycitrate upon the accumulation of lipid in the rat. I. lipogenesis. Lipids 1974;9:121–8.
[14] Sullivan AC, Triscari J, Hamilton JG, Miller ON. Effect of (–)-hydroxycitrate upon the accumulation of lipid in the rat. II. appetite. Lipids 1974;9:129–34.
[15] Triscari J, Sullivan AC. Anti-obesity activity of a novel lipid synthesis inhibitor. Int J Obes 1984;8:227–39.
[16] Ramos RR, Saenz JLS, Aguilar RJA. Extract of *Garcinia cambogia* in controlling obesity. Investigacion Medica Internacional 1995;22:97–100.
[17] Mattes DR, Bormann L. Effects of (–)-Hydroxycitric Acid on Appetitive Variables. Physiol Behav 2000; 71:87–94.
[18] Leonhardt M, Hrupka B, Langhans W. Effect of hydroxycitrate on food intake and body weight regain after a period of restrictive feeding in male rats. Physiol Behav 2001;74:191–6.
[19] Westerterp-Plantenga MS, Kovacs EMR. The effect of (–)-hydroxycitrate on energy intake and satiety in overweight humans. Int J Obes 2002;26:870–2.
[20] Shara, M, Ohia, SE, Yasmin, T, Zardetto-Smith, A, Kincaid, A, Bagchi, M, Chatterjee, A, Bagchi, D, Stohs, SJ. Dose- and time-dependent effects of a novel (–)-hydroxycitric acid extract on body weight, hepatic and testicular lipid peroxidation, DNA fragmentation and histopathological data over a period of 90 days. Mol Cell Biochem (in press).
[21] Ohia SE, Opere CA, LeDay AM, Bagchi M, Bagchi D, Stohs SJ. Safety and mechanism of appetite suppression by a novel hydroxycitric acid extract (HCA-SX). Mol Cell Biochem 2002;238:89–103.
[22] Loe YC, Bergeron N, Rodriguez N, Schwarz JM. Gas chromatography/mass spectrometry method to quantify blood hydroxycitrate concentration. Anal Biochem 2001;292:148–54.
[23] Crawford V, Scheckenbach R, Preuss HG. Effects of niacin-bound chromium supplementation on body composition in overweight African-American women. Diab Obes Metab 1999;1:331–7.
[24] Grant KE, Chandler RM, Castle AL, Ivy JL. Chromium and exercise training: effect on obese women. Med Sci Sports Exer 1997;29:992–98.
[25] Preuss HG, Montamarry S, Echard B, Scheckenbach R, Bagchi D. Long-term effects of chromium, grape seed extract, and zinc on various metabolic parameters of rats. Mol Cell Biochem 2001;223:95–102.
[26] Prakash AO, Mathur S, Mathur R. Effect of feeding *Gymnema sylvestre* leaves on blood glucose in beryllium nitrate treated rats. J Ethnopharmacol 1986;18:143–6.

[27] Ninomiya Y, Imoto T. Gurmarin inhibition of sweet taste responses in mice. Am J Physiol 1995;268: R1019–25.
[28] Preuss HG, Jarrell ST, Scheckenbach R, Lieberman S, Anderson RA. Comparative effects of chromium, vanadium and *Gymnema sylvestre* on sugar-induced blood pressure elevations in SHR. J Am Coll Nutr 1998;17:116–23.
[29] Shanmugasundaram ERB, Rajeswari G, Baskaran K, Kumar BRR, Shanmugasundaram KR, Ahmath BK. Use of *Gymnema sylvestre* leaf extract in the control of blood in insulin-dependent diabetes mellitus. J Ethnopharmacol 1990;30:281–94.
[30] Ohia SE, Awe O, LeDay AM, Opere CA, Bagchi D. Effect of hydroxycitric acid on serotonin release from isolated rat brain cortex. Res Commun Mol Pathol Pharmacol 2001;109:210–6.
[31] Shara MA, Dickson PH, Bagchi D, Stohs SJ. Excretion of formaldehyde, malondialdehyde, acetaldehyde and acetone in the urine of rats in response to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, paraquat, endrin and carbon tetrachloride. J Chromatogr 1992;576:221–33.
[32] Committee for Proprietary Medicinal Products. Note for Guidance on Clinical Investigation of Drugs Used in Weight Control. London, England: The European Agency for the Evaluation of Medical Products; 1997.
[33] Montgomery DC. Design and analysis of experiments, 5th ed. New York: John Wiley & Sons, Inc., 2001.
[34] Littell RC, Stroup WW, Freund RJ. SAS for linear models, 4th ed. Cary, NC: SAS Institute Inc, 2002.
[35] CNN.com.Health. Report: Dietary supplement warning system lacking. Available at www.cnn.com. Accessed February 25, 2003.
[36] Heymsfield SB, Allison DB, Vasselli JR, Pietrobelli A, Greenfield D, Nunez C. Garcinia cambogia (hydroxycitric acid) as a potential antiobesity agent: a randomized controlled trial. JAMA 1998;280:1596–600.
[37] Dagogo-Jack S. Human leptin regulation and promise in pharmacotherapy. Curr Drug Targets 2001;2:181–95.
[38] Adeyemi E, Abdulle A. A comparison of plasma leptin levels in obese and lean individuals in the United Arab emirates. Nutr Res 2000;20:157–66.
[39] Lerario DDG, Ferreira SRG, Miranda WL, Chacra AR. Influence of dexamethasone and weight loss on the regulation of serum leptin levels in obese individuals. Braz J Med Biol Res 2001;34:479–87.
[40] Levy JR, Gyarmati J, Lesko JM, Adler RA, Stevens W. Dual regulation of leptin secretion: intracellular energy and calcium dependence of regulated pathway. Am J Physiol Endocrinol Metab 2000;278:E892–901.
[41] Leibowitz SF, Alexander JT. Hypothalamic serotonin in control of eating behavior, meal size, and body weight. Biol Psychiatry 1988;44:851–64.
[42] Casper RC. Serotonin, a major player in the regulation of feeding and affect. Biol Psychiatry 1998;44:795–7.
[43] Dhanakoti SN, Draper HH. Response of urinary malondialdehyde to factors that stimulate lipid peroxidation *in vivo*. Lipids 1987;22:643–6.
[44] Foster DW. Diabetes mellitus. Braunwald E, Isselbacher KJ, Petersdorf RG, Wilson JD, Martin JB, Fauci AS, editors. Harrison's Principles of Internal Medicine. 11th. New York, NY: McGraw-Hill, 1987. 1778–96.
[45] Winters DK, Clejan LA, Cederbaum AI. Oxidation of glycerol to formaldehyde by rat liver microsomes. Biochem Biophy Res Commun 1988;153:612–7.
[46] Winters DK, Cederbaum AI. Oxidation of glycerol to formaldehyde by rat liver microsomes. Effects of cytochrome P-450 inducing agents. Biochem Pharmacol 1990;39:697–705.

British Journal of Nutrition (2005), 94, 432–436

DOI: 10.1079/BJN20051502

Effects of encapsulated green tea and Guarana extracts containing a mixture of epigallocatechin-3-gallate and caffeine on 24 h energy expenditure and fat oxidation in men

Sonia Bérubé-Parent, Catherine Pelletier, Jean Doré and Angelo Tremblay*

Division of Kinesiology, Laval University, Ste-Foy, Québec, Canada, G1K 7P4

(Received 7 October 2004 – Revised 8 April 2005 – Accepted 11 April 2005)

It has been reported that green tea has a thermogenic effect, due to its caffeine content and probably also to the catechin, epigallocatechin-3-gallate (EGCG). The main aim of the present study was to compare the effect of a mixture of green tea and Guarana extracts containing a fixed dose of caffeine and variable doses of EGCG on 24 h energy expenditure and fat oxidation. Fourteen subjects took part to this randomized, placebo-controlled, double-blind, cross-over study. Each subject was tested five times in a metabolic chamber to measure 24 h energy expenditure, substrate oxidation and blood pressure. During each stay, the subjects ingested a capsule of placebo or capsules containing 200 mg caffeine and a variable dose of EGCG (90, 200, 300 or 400 mg) three times daily, 30 min before standardized meals. Twenty-four hour energy expenditure increased significantly by about 750 kJ with all EGCG–caffeine mixtures compared with placebo. No effect of the EGCG–caffeine mixture was observed for lipid oxidation. Systolic and diastolic blood pressure increased by about 7 and 5 mmHg, respectively, with the EGCG–caffeine mixtures compared with placebo. This increase was significant only for 24 h diastolic blood pressure. The main finding of the study was the increase in 24 h energy expenditure with the EGCG–caffeine mixtures. However, this increase was similar with all doses of EGCG in the mixtures.

Green tea: Body weight: Energy balance

Green tea is one of the most widely consumed beverages in the world and is currently perceived as a healthy drink. Green tea contains a large amount of catechins (30 to 42 % dry weight), a group of very active flavonoids (Yang & Landau, 2000; Dusfresne & Farnworth, 2001). The catechins, which are antioxidants, have been attributed beneficial health properties such as protection against CVD and certain types of cancer. Also, some attention has recently been given to the possible beneficial effects of green tea on the treatment of obesity.

The catechins epicatechin, epicatechin-3-gallate, epigallocatechin and epigallocatechin-3-gallate (EGCG) are the major components of green tea leaves. EGCG is the most abundant catechin and has received the most attention (Yang & Landau, 2000). Caffeine represents approximately 3 to 5 % of the dry weight of green tea (Yang & Landau, 2000; Dusfresne & Farnworth, 2001). Caffeine consumption has been related to an increase in energy expenditure (Astrup *et al.* 1990; Dulloo *et al.* 1989), which explains why the thermogenic effect of green tea is generally attributed to its caffeine content. However, Dulloo *et al.* (2000) reported that, in rats, a green tea extract stimulates brown adipose tissue thermogenesis to a much greater extent than that which can be attributed to its caffeine content *per se*. In another study, ten healthy men were assigned to three treatments: green tea extract containing 50 mg caffeine and 90 mg EGCG, caffeine (50 mg) or placebo. A capsule of green tea extract, caffeine or placebo was taken with each meal. Ingestion of green tea extract increased 24 h energy expenditure by 4 % (328 kJ), reflecting its stimulatory effect on thermogenesis. The study also found a reduction in RQ during the green tea extract treatment, suggesting an increase in fat oxidation (Dulloo *et al.* 1999). On the other hand, the caffeine treatment did not produce any effect on these variables.

In addition, the thermogenesis and fat oxidation stimulation obtained in that study was not accompanied by an increase in heart rate that may be seen when patients are treated with sympathomimetic anti-obesity drugs. Since obese individuals are at greater risk of developing cardiac problems, the increase in heart rate and blood pressure frequently observed when treated with sympathomimetic agents is a matter of concern for health professionals. In this context, the green tea extract and caffeine mixture seems to have potential as an effective alternative to these anti-obesity drugs.

Since it was shown that a total daily dose of 270 mg (3 × 90 mg) EGCG combined with a total daily dose of 150 mg (3 × 50 mg) caffeine has the potential to produce an increase in energy expenditure, augmenting the amount of caffeine in the blend could possibly accentuate this increase. Moreover, augmenting the amount of EGCG in the green tea extract mixture may produce a greater increase in energy expenditure. If so, the most effective level of EGCG to use in combination with caffeine to produce a significant increase in energy expenditure and fat oxidation without producing negative cardio-stimulatory side-effects would deserve specific investigation.

Abbreviation: EGCG, epigallocatechin-3-gallate.

* Corresponding author: Dr Angelo Tremblay, fax +1 418 656 3044, email angelo.tremblay@kin.msp.ulaval.

Therefore, the first aim of the present study was to assess the impact of four mixtures of green tea and Guarana (a plant that contains caffeine) extracts containing a fixed 600 mg daily (3 × 200 mg) dose of caffeine and different amounts of EGCG (270 mg/d: 3 × 90 mg; 600 mg/d: 3 × 200 mg; 900 mg/d: 3 × 300 mg; 1200 mg/d: 3 × 400 mg) on 24 h energy expenditure, RQ and substrate oxidation in comparison with a placebo. The second aim of the study was to determine whether there is a dose-related effect of EGCG, and if so, which dose produces a greater increase in energy expenditure and fat oxidation without inducing significant cardio-stimulatory effects when combined with caffeine.

Materials and methods

Subjects

Healthy, non-smoking and sedentary men (n 14), from 20 to 50 years of age and with BMI between 20 and 27 kg/m^2, were selected to participate in the study (Table 1). Subjects on a particular diet (vegetarian), subjects consuming a diet rich in capsaicin (e.g. red pepper), subjects using anorectic or related compounds (sympathomimetic compounds), athletes or regularly active individuals (>30 min of intense physical activity three times weekly) and subjects with a caffeine intake >200 mg/d (about two small cups of coffee daily) were all excluded from the study. In addition, all fourteen participants had a stable weight (±3 kg) for at least 3 months before the protocol and no history of weight loss (≥4·5 kg). They gave their written consent to participate in this study, which received approval of the Laval University Medical Ethics Committee.

Study design and randomization

The present study has a randomized, placebo-controlled, double-blind, cross-over design. Subjects came to the laboratory for a first visit during which anthropometric and metabolic rate measurements were performed. Each subject then spent 24 h in a metabolic chamber on five separate occasions and was randomly assigned to receive one of the following five treatments, three times daily:

(1) Mixture of green tea and Guarana extracts: 200 mg caffeine (600 mg/d) + 90 mg EGCG (270 mg/d);
(2) Mixture of green tea and Guarana extracts: 200 mg caffeine (600 mg/d) + 200 mg EGCG (600 mg/d);
(3) Mixture of green tea and Guarana extracts: 200 mg caffeine (600 mg/d) + 300 mg EGCG (900 mg/d);
(4) Mixture of green tea and Guarana extracts: 200 mg caffeine (600 mg/d) + 400 mg EGCG (1200 mg/d);
(5) Placebo: inert filler of cellulose.

Table 1. Characteristics of the subjects

(Mean values with their standard deviation for fourteen subjects)

	Mean	SD
Age (years)	34·7	8·6
Weight (kg)	78·6	12·9
BMI (kg/m^2)	25·7	2·7
% Body fat	19·9	7·8
Fat mass (kg)	16·0	7·0
Fat-free mass (kg)	62·7	10·0

The capsules of the four pre-existing mixtures and placebo were developed and standardized by Invate Health Sciences Research Inc. (previously known as Muscle Tech Research and Development; Mississauga, Ontario, Canada). The four mixtures contained a green tea extract in which EGCG represented 45 % dry weight. Therefore these mixtures also contained unknown amounts of other catechins and possibly caffeine. The mixtures were also composed of a Guarana extract that contained caffeine and other possibly unknown components. The amounts of green tea and Guarana extracts were adjusted to obtain the desired dose of EGCG (90, 200, 300 and 400 mg/mixture) and caffeine (fixed dose of 200 mg for each mixture).

Subjects received all of the treatments but in a different order depending on the randomization to which they were assigned. The first dose was given at 08.00 hours, followed by the standardized breakfast 30 min later. The second dose was at 12.00 hours, followed by a standardized lunch 30 min later. The third dose was at 18.00 hours, followed by a standardized dinner 30 min later. Before each standardized meal, immediately after and every hour until the next meal, levels of hunger and satiety were evaluated with visual analogue scales. At each metabolic chamber visit, the 24 h ambulatory blood pressure monitor was installed. The 24 h urine collection was also performed at these visits. It is to be noted that subjects did not change their diet or activity pattern during the study. The various sessions in the metabolic chamber were administered within an interval of 5–10 d of each other for each subject.

Measurements

Anthropometric measurements. Body weight was taken with a standard beam scale. Waist and hip circumferences were taken according to Harrison *et al.* (1988). Body density was determined by hydrodensitometry (Behnke & Wilmore, 1974). The closed-circuit He dilution method (Meneely & Kaltreider, 1949) was used to assess residual lung volume. The Siri formula (Siri, 1956) was then used to estimate the percentage of body fat from body density, while fat mass and fat-free mass were calculated from the derived percentage of body fat and total body weight.

Measurement of resting metabolic rate and substrate oxidation. Resting energy expenditure (RMR) was measured by indirect calorimetry after a 12 h overnight fast. After resting for 15 min, expired gas collection was performed through a mouthpiece for 15 min while the nose was clipped during the whole measurement. O_2 and CO_2 concentrations were determined by nondispersive IR analysis (Uras 10 E; Hartmann & Braun, Frankfurt, Germany) whereas pulmonary ventilation determination was assessed with an S430A measurement system (KL Engineering, Ventura, CA, USA). The Weir formula (Weir, 1949) was used to determine the energy equivalent of O_2 volume. The determination of substrate oxidation was assessed through the calculations previously described by Frayn (1983) while assuming that protein oxidation contributes 10 % of total energy expenditure measured under these conditions.

Measurement of 24 h energy expenditure and substrate oxidation. Twenty-four hour total energy expenditure was measured with a whole-body indirect calorimeter, which has

been shown to provide highly reproducible data in our laboratory (White *et al.* 1996). Subjects entered the calorimeter at about 07.30 hours after an overnight fast (12 h). During this stay, subjects were maintained in energy balance by using the resting energy expenditure performed at the initial visit and by extrapolating this value over a 24 h period and then multiplying this value by an activity factor of 1·32 (White *et al.* 1997). The same energy intake was maintained for the five measurements of 24 h energy expenditure. Moreover, the nutrient composition of the diet (daily food quotient 0·85), the sedentary life-style pattern (watching television, computer, reading, etc.) and the meal pattern, as well as the period of sleep were also standardized in each session. It was not permitted to eat or drink any other foods than those provided and therefore no foods or beverages containing caffeine were allowed during the metabolic chamber stay. Before each metabolic chamber visit, subjects were asked to refrain from exercise and eliminate consumption of foods or beverages containing caffeine for 24 h prior to the measurements.

Twenty-four hour blood pressure and heart rate monitoring. To determine the 24 h means (overnight + daily) for systolic and diastolic blood pressure and heart rate, subjects were asked to wear an ambulatory blood pressure monitor in the metabolic chamber. The ambulatory blood pressure device (model #90 207; Space Labs Medical, Redmond, WA, USA), which was installed by the investigator, consists of a programmed console that is worn on the belt with an appropriate size cuff (depending on arm circumference) worn on the non-dominant arm and a cable connecting the console to the cuff. Data were recorded at frequent intervals throughout the day (every 30 min from 08.00 to 22.00 hours) and at night (every hour from 22.00 to 08.00 hours) and were then analysed with a computerized system (FT 1000A).

Twenty-four hour urine collection (urinary nitrogen and catecholamine excretion). While in the metabolic chamber (five visits), patients were asked to collect urine for a 24 h period and a sample was taken for analysis to measure urinary N and catecholamine excretion. The extraction and separation of urinary catecholamines was done using C_{18} solid-phase extraction sorbent and HPLC (Talwar *et al.* 2002).

Levels of hunger and satiety. The levels of hunger and satiety were evaluated with visual analogue scales. Before the standardized breakfast, levels of hunger and satiety were evaluated and the measures were repeated immediately after and 60, 120 and 180 min after the breakfast. The same pattern was used for the standardized lunch and dinner.

Statistical analysis

JUMP Software 3.1.6.2 (SAS Institute Inc., Cary, NC, USA) was used for all analyses. ANOVA for repeated measures was performed to determine if there were differences between the effects of the five treatments on 24 h energy expenditure, sleeping metabolic rate, RQ, carbohydrate oxidation, lipid oxidation, heart rate, systolic and diastolic blood pressure (24 h, day and night), and noradrenaline, adrenaline and dopamine excretion. When ANOVA was significant ($P<0·05$), paired t tests were performed to compare each pair of treatments in order to detect the treatments between which there were the differences. As there were multiple comparisons, Bonferonni correction was applied and results of the paired t tests were considered statistically significant at $P<0·005$ (ten comparisons). The effects of the five treatments on visual analogue scale ratings were also determined by an ANOVA for repeated measures. Fasting ratings (before breakfast), area under the curve for the entire day (from after breakfast to 240 min after dinner) and mean rating for the three hours following each meal were compared. Results are presented as means with their standard deviation.

Results

Within all the variables measured, the ANOVA for repeated measures revealed that the EGCG–caffeine mixtures had an effect on 24 h energy expenditure, 24 h diastolic blood pressure and carbohydrate oxidation (Table 2). Paired t tests were then performed on these three variables to determine between which treatments there were differences. Table 2 also indicates that the EGCG–caffeine mixtures favoured an increase in sleeping metabolic rate and 24 h systolic blood pressure but the effect

Table 2. Effect of treatment on the variables measured in the metabolic chamber
(Mean values with their standard deviation for fourteen subjects)

	Placebo		270 mg EGCG/d (3 × 90 mg)		600 mg EGCG/d (3 × 200 mg)		900 mg EGCG/d (3 × 300 mg)		1200 mg EGCG/d (3 × 400 mg)	
Variable	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
24 h energy expenditure (kJ/d)**	9421	1446	10 081	1585	10 134	1435	10 209	1705	10 249	1716
Sleeping metabolic rate (kJ)	7136	1181	7321	1094	7464	1156	7542	1195	7827	1330
24 h RQ	0·89	0·03	0·87	0·05	0·91	0·05	0·90	0·08	0·86	0·04
Carbohydrate oxidation (g/d)*	352·9	59·9	334·2	100·4	404·5	87·2	396·4	149·3	327·3	104·5
Lipid oxidation (g/d)	63·2	34·3	83·6	48·5	59·2	45·8	65·9	70·7	94·3	43·4
24 h heart rate (beats/min)	63	9	63	9	62	7	63	10	66	10
24 h systolic blood pressure (mmHg)	116	6	122	8	123	8	123	8	123	7
24 h diastolic blood pressure (mmHg)*	70	4	74	4	75	5	75	5	75	5
Noradrenaline excretion (nmol/d)	216	117	241	87	211	98	231	98	222	93
Adrenaline excretion (nmol/d)	<90	37	<104	29	<124	32	<110	36	<94	33
Dopamine excretion (nmol/d)	1950	871	1937	615	1856	586	1510	650	1674	583

EGCG, epigallocatechin-3-gallate.
Statistically significant effect of treatment (ANOVA for repeated measures for difference between groups): *$P<0·05$, **$P<0·001$.

did not reach standard statistical significance. Thus, no *a posteriori* comparison was performed for these two variables.

The EGCG–caffeine mixtures increased 24 h energy expenditure by about 750 kJ (8 %) compared with placebo (Fig. 1). Increasing the dose of EGCG in the mixtures induced a mild increase in 24 h energy expenditure, but these differences were not significant, even between the lowest (270 mg/d: 3 × 90 mg) and the highest (1200 mg/d: 3 × 400 mg) doses. On the other hand, the paired *t* tests for carbohydrate oxidation did not reveal an *a posteriori* significant difference for any pair of treatments. Contrary to what was expected, the intake of EGCG–caffeine mixtures had no effect on RQ, lipid oxidation or catecholamine excretion.

As the different doses of EGCG induced the same increase in blood pressure for the day, the night and for the entire 24 h period, only the 24 h results are presented. Twenty-four hour systolic blood pressure was increased by about 7 mmHg, independently of the dose of EGCG (Fig. 2(a)). However, the effect of the EGCG–caffeine mixtures on 24 h systolic blood pressure did not reach significance. Twenty-four hour diastolic blood pressure was increased by about 5 mmHg by the EGCG–caffeine mixtures compared with placebo (Fig. 2(b)). The paired *t* test revealed a significant difference between placebo and the 270, 600 and 900 mg daily doses of EGCG, but there was no difference between placebo and the 1200 mg daily dose of EGCG or between the different doses.

Fasting visual analogue scale ratings did not differ between the five visits of the subjects in the metabolic chamber. The intake of EGCG–caffeine mixtures did not modify the response levels of hunger or satiety of the visual analogue scale, either for the entire day or for each meal analysed separately (results not shown).

Discussion

The objective of the present study was to investigate the impact of the mixture of green tea and Guarana extracts on energy metabolism with a design focused on a clinical outcome, but not intended to discriminate the independent effect of each extract. In fact, the main preoccupation in this study was to verify the possibility that increasing the EGCG content of a compound containing a fixed dose of caffeine (Guarana extracts) and EGCG (green tea extracts)

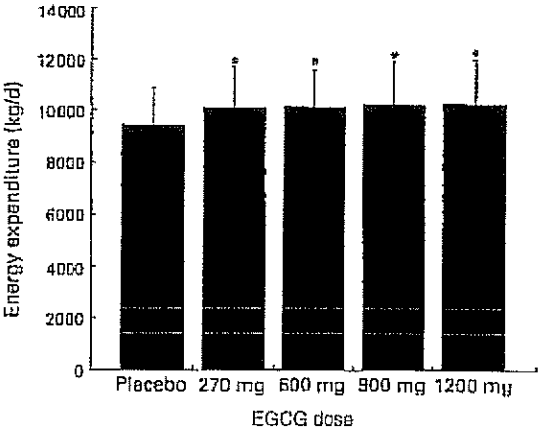


Fig. 1. Twenty-four hour energy expenditure with the placebo and the epigallocatechin-3-gallate (EGCG)–caffeine mixtures containing 200 mg caffeine (600 mg/d) and different daily doses of EGCG. Values are means with their standard deviation shown by vertical bars. Mean values were significantly different from placebo (paired *t* test with Bonferonni correction): *$P<0.005$.

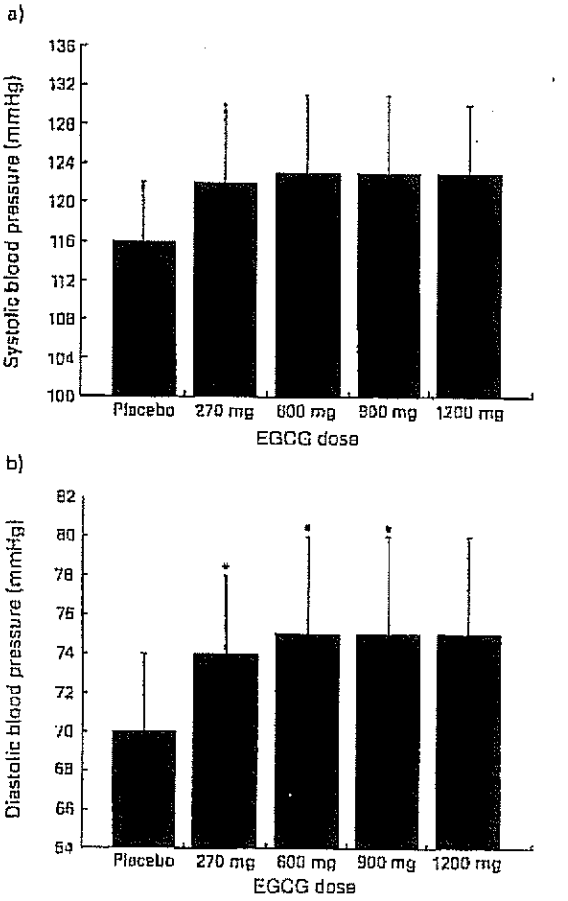


Fig. 2. Twenty-four hour systolic (a) and diastolic (b) blood pressure with the placebo and the epigallocatechin-3-gallate (EGCG)–caffeine mixtures containing 200 mg caffeine (600 mg/d) and different daily doses of EGCG. Values are means with their standard deviation shown by vertical bars. Mean values were significantly different from placebo (paired *t* test with Bonferonni correction): *$P<0.005$.

enhances the impact of the compound on 24 h energy expenditure. As expected, the four different EGCG–caffeine mixtures tested in the study induced a significant increase in 24 h energy expenditure compared with the placebo. There were no statistically differences among the EGCG–caffeine mixtures with the different amounts of EGCG. Indeed, the difference between the highest (1200 mg/d: 3 × 400 mg) and lowest (270 mg/d: 3 × 90 mg) doses reached a difference of only about 168 kJ. In this regard, our results are clear and innovative in that they demonstrate that, beyond a certain threshold, the EGCG content of a compound only produces a small non-significant additional increase in 24 h energy expenditure. Therefore, from a clinical standpoint it does not appear relevant to increase the EGCG content of the reference mixture in order to produce a substantial increase in daily energy expenditure.

No effects of the EGCG–caffeine mixtures on RQ and macronutrient oxidation were observed. This is possibly related to the high variability observed in the results with the different doses of EGCG. This absence of effect of the EGCG–caffeine mixture on RQ is concordant with the results obtained by Kovacs *et al.* (2004) during weight maintenance with green tea (323 mg/d: about 108 mg EGCG three times daily; 104 mg/d: about 35 mg

caffeine three times daily) after weight loss. However, when comparing the EGCG–caffeine mixture with the lowest dose of EGCG (90 mg) with the placebo, there appears to be a decrease in RQ and an increase in lipid oxidation. This is in accordance with results obtained with the same EGCG dose in the study by Dulloo *et al.* (1999). Therefore, it could be hypothesized that the 90 mg (270 mg/d) EGCG dose is the optimal concentration to produce an effect on macronutrient oxidation. It is to be noted that substantial fluctuations in RQ were observed in the present study, which is concordant with the fact that RQ is a less stable variable than energy expenditure and is characterized by a lower reproducibility than 24 h energy expenditure (White *et al.* 1996). In this regard, we cannot exclude the possibility that increased fluctuation in 24 h RQ might have prevented the demonstration of a significant EGCG effect.

The EGCG–caffeine mixtures did not produce significant increases in heart rate as was observed in the Dulloo *et al.* (1999) study. However, a non-significant increase in 24 h systolic blood pressure accompanied by a significant increase in 24 h diastolic blood pressure was observed. It is possible that the EGCG–caffeine treatment used by Dulloo *et al.* (1999) produced a slight increase in blood pressure even if there was no change in heart rate. However, blood pressure measurements were not tested and/or presented in that study. Since regular physical activity results in a decrease in resting heart rate and blood pressure (Seals & Hagberg, 1984; McArdle *et al.* 1996), we can suppose that adding regular exercise when taking the EGCG–caffeine mixture could be helpful to prevent the slight cardio-stimulatory effects produced by this mixture. This beneficial effect has been observed with the weight-loss medication Meridia™, which is known to produce increases in blood pressure and heart rate. Indeed, it was shown that combining physical activity with Meridia™ prevented the cardio-stimulatory effects that were observed when the drug was combined with diet alone (Bérubé-Parent *et al.* 2001).

The EGCG–caffeine mixture should be considered as a good complement in a weight-loss programme. Indeed, this EGCG–caffeine mixture appears to have potential benefits in the treatment of obesity and should be further tested in a clinical context where nutrition counselling and supervision are offered with regular physical activity participation. Such an approach would be expected to attenuate the decrease in energy expenditure related to body-weight loss while preventing cardio-stimulating effects.

Acknowledgements

This research was supported by Iovate Health Sciences Research Inc. A. T. is partly supported by the Canada Research Chair in Physical Activity, Nutrition and Energy Balance.

References

Astrup A, Toubro S, Cannon S, Hein P, Breum L & Madsen J (1990) Caffeine: a double-blind, placebo-controlled study of its thermogenic, metabolic, and cardiovascular effects in healthy volunteers. *Am J Clin Nutr* 51, 759–767.

Behnke AR & Wilmore JH (1974) *Evaluation and Regulation of Body Build and Composition*. pp. 20–37. Englewood Cliffs, NJ: Prentice-Hall.

Bérubé-Parent S, St-Pierre S, Prud'homme D, Doucet E & Tremblay A (2001) Obesity treatment with a progressive clinical tri-therapy combining sibutramine and a supervised diet–exercise intervention. *Int J Obesity Relat Metab Disord* 25, 1144–1153.

Dulloo AG, Geissler CA, Horton T, Collins A & Miller DS (1989) Normal caffeine consumption: influence on thermogenesis and daily energy expenditure in lean and postobese human volunteers. *Am J Clin Nutr* 49, 44–50.

Dulloo A, Duret C, Girardier L, Mensi N, Fathi M, Chantre P & Vandermander J (1999) Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans. *Am J Clin Nutr* 70, 1040–1045.

Dulloo A, Seydoux J, Girardier L, Chantre P & Vandermander J (2000) Green tea and thermogenesis: interactions between catechin-polyphenols, caffeine and sympathetic activity. *Int J Obesity Relat Metab Disord* 24, 252–258.

Dufresne CJ & Farnworth ER (2001) A review of latest research findings on the health promotion properties of tea. *J Nutr Biochem* 12, 404–421.

Frayn K (1983) Calculation of substrate oxidation rates *in vivo* from gaseous exchange. *J Appl Physiol* 55, 628–634.

Harrison GG, Buskirk ER, Carter JEL, Johnston FE, Lohman TG, Pollock ML, Roche AF & Wilmore J (1988) Skinfold thickness and measurement Technique. In *Anthropometric Standardization Reference Manual*. pp. 55–80 [TG Lohman, AF Roche and R Martorell, editors]. Champaign, IL: Human Kinetics Books.

Kovacs EM, Lejeune MP, Nijs I & Westerterp-Plantenga MS (2004) Effects of green tea on weight maintenance after body-weight loss. *Br J Nutr* 91, 431–437.

McArdle WD, Katch FI & Katch VL (1996) Functional capacity of the cardiovascular system. In *Exercise Physiology*. 4th ed., pp. 296–312 [D Halliday, editor]. Baltimore, MD: William and Wilkins.

Meneely EA & Kaltreider NL (1949) Volume of the lung determined by helium dilution. *J Clin Invest* 28, 129–139.

Seals DR & Hagberg JM (1984) The effect of exercise training on human hypertension. *Med Sci Sports Exerc* 16, 207–215.

Siri WE (1956) The gross composition of the body. *Adv Biol Med Physiol* 4, 238–280.

Talwar D, Williamson C, McLaughlin A, Gill A & O'Reilly DS (2002) Extraction and separation of urinary catecholamines as their diphenyl boronate complexes using C_{18} solid-phase extraction sorbent and high-performance liquid chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 769, 341–349.

Weir JB (1949) New method for calculating metabolic rate with special references to protein metabolism. *J Physiol (Lond)* 109, 1–9.

White MD, Bouchard G, Buemann B, Almeras N, Despres JP, Bouchard C & Tremblay A (1996) Reproducibility of 24-h energy expenditure and macronutrient oxidation rates in an indirect calorimeter. *J Appl Physiol* 80, 133–139.

White MD, Bouchard G, Buemann B, Despres JP, Bouchard C & Tremblay A (1997) Energy and macronutrient balances for humans in a whole body metabolic chamber without control of preceding diet and activity level. *Int J Obes Relat Metab Disord* 21, 135–140.

Yang C & Landau J (2000) Effects of tea consumption on nutrition and health. *J Nutr* 130, 2409–2412.

Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans[1–3]

Abdul G Dulloo, Claudette Duret, Dorothée Rohrer, Lucien Girardier, Nouri Mensi, Marc Fathi, Philippe Chantre, and Jacques Vandermander

ABSTRACT

Background: Current interest in the role of functional foods in weight control has focused on plant ingredients capable of interfering with the sympathoadrenal system.

Objective: We investigated whether a green tea extract, by virtue of its high content of caffeine and catechin polyphenols, could increase 24-h energy expenditure (EE) and fat oxidation in humans.

Design: Twenty-four–hour EE, the respiratory quotient (RQ), and the urinary excretion of nitrogen and catecholamines were measured in a respiratory chamber in 10 healthy men. On 3 separate occasions, subjects were randomly assigned among 3 treatments: green tea extract (50 mg caffeine and 90 mg epigallocatechin gallate), caffeine (50 mg), and placebo, which they ingested at breakfast, lunch, and dinner.

Results: Relative to placebo, treatment with the green tea extract resulted in a significant increase in 24-h EE (4%; $P < 0.01$) and a significant decrease in 24-h RQ (from 0.88 to 0.85; $P < 0.001$) without any change in urinary nitrogen. Twenty-four–hour urinary norepinephrine excretion was higher during treatment with the green tea extract than with the placebo (40%, $P < 0.05$). Treatment with caffeine in amounts equivalent to those found in the green tea extract had no effect on EE and RQ nor on urinary nitrogen or catecholamines.

Conclusions: Green tea has thermogenic properties and promotes fat oxidation beyond that explained by its caffeine content per se. The green tea extract may play a role in the control of body composition via sympathetic activation of thermogenesis, fat oxidation, or both. *Am J Clin Nutr* 1999;70:1040–5.

KEY WORDS Obesity, thermogenesis, catechins, polyphenols, caffeine, sympathetic nervous system, green tea, fat oxidation, catecholamines, men

INTRODUCTION

Fundamentally, there are only 2 ways to treat obesity: reduce energy intake or increase energy expenditure (EE). Because thermogenesis and fat oxidation are to a large extent under the control of the sympathetic nervous system (SNS), approaches that mimic or interfere with the SNS and its neurotransmitter norepinephrine offer a rational approach for obesity management (1–3). In this context, there has been renewed interest in the potential thermogenic effects of many compounds extracted from plants (eg, caffeine from coffee and tea, ephedrine from ephedra, and capsaicin from pungent spices), largely because of their potential to modulate catecholamine release and activity (4) . For example, capsaicin-rich foods (eg, chili peppers and red peppers) have been shown to stimulate fat oxidation and thermogenesis in humans (5, 6), and caffeine in relatively small amounts can potentiate thermogenesis induced by sympathetic stimuli, whether in response to cold, moderate exercise, or sympathomimetic drugs like ephedrine (7). In fact, long-term clinical trials have shown greater losses in body weight and body fat in obese patients treated with a combination of caffeine and ephedrine than in those treated with placebo, caffeine, or ephedrine alone (8).

Previous work in our laboratory, in which an in vitro system was used to measure the respiration rate of brown adipose tissue in rats, suggests that the interaction between caffeine and ephedrine resides in ephedrine's induced enhancement of sympathetic neural release of norepinephrine together with caffeine's ability to inhibit the phosphodiesterase-induced degradation of intracellular cyclic AMP (cAMP), and, to a lesser extent, caffeine's antagonism of the negative modulatory effect of adenosine on increased norepinephrine release (9). The net result, therefore, would be an elevated cellular concentration of cAMP—a critical intracellular mediator for the actions of catecholamines on thermogenesis. Apart from phosphodiesterases, adenosine, and certain prostaglandins, the concentration of norepinephrine at the synaptic junction and its interaction with adrenoceptors is also likely to be negatively modulated through its enzymatic degradation, namely by catechol *O*-methyltransferase (COMT) (10). Given

[1] From the Department of Physiology, Faculty of Medicine, University of Geneva; Geneva University Hospital; and Laboratoires Arkopharma, Nice, France.

[2] Supported in part by Arkopharma Laboratories and by the Swiss National Science Research Fund.

[3] Address reprint requests to AG Dulloo, Institute of Physiology, University of Fribourg, Rue de Musée 5, CH-1700 Fribourg, Switzerland. E-mail: abdul.dulloo@unifr.ch.

Received December 16, 1998.

Accepted for publication March 31, 1999.

Am J Clin Nutr 1999;70:1040–5. Printed in USA.

evidence that this enzyme can be inhibited by certain tea polyphenols (11), we recently investigated in our in vitro system whether an extract of green tea, by virtue of its high content of both caffeine and catechin polyphenols, could be an effective promoter of thermogenesis. These in vitro results (12) can be summarized as follows: *1*) the green tea extract was found to be more effective than were equivalent amounts of caffeine in stimulating peripheral tissue thermogenesis, and *2*) this difference between the green tea extract and equimolar caffeine in activating thermogenesis was much more marked under conditions of increased norepinephrine release because the synergistic interaction between the green tea extract and ephedrine on tissue thermogenesis was much more pronounced than that of caffeine or ephedrine.

On the basis of these in vitro data, our main objectives in this study were 2-fold: *1*) to examine the extent to which daily administration of capsules containing a green tea extract (containing catechin polyphenols and caffeine in amounts comparable with those commonly consumed in green tea beverages in Asian communities) would stimulate thermogenesis and increase daily EE in humans, and *2*) to determine whether the effects of the green tea extract on the metabolic rate and substrate oxidation in humans would be greater than that explained by its caffeine content per se.

SUBJECTS AND METHODS

Subjects

Healthy young men were recruited from the student and staff population of our University after complete medical and nutritional histories were obtained by use of a questionnaire. Smokers, competitive athletes, and persons who engaged in intense physical activities or who had a history of weight loss were not eligible for inclusion in the study. Inclusion criteria included body fatnesses ranging from lean to mildly obese (8-30% body fat). All selected subjects habitually consumed a typical Western diet, with fat contributing 35–40% of dietary energy intake, and their estimated intake of methylxanthines (mostly as caffeine-containing beverages) ranged from 100 to 200 mg/d. At the onset of the study, body weight and height were measured and body fat was determined by the method of Durnin and Womersley (13) from measurements of skinfold thicknesses taken at 4 sites with a Harpenden skinfold caliper (British Indicators, Ltd, London); fat-free-mass (FFM) was calculated as the difference between body weight and body fat. Mean (±SEM) values for some of the physical characteristics of the 10 men participating in this study were as follows: age, 25 ± 1 y; height, 177 ± 3 cm; weight, 78.7 ± 4.3 kg; body mass index (BMI; in kg/m^2), 25.1 ± 1.2; percentage body fat, 18.2 ± 1.8%; and FFM, 63.8 ± 2.5 kg. The study was approved by the Ethical Committee for Human Experimentation of the University of Geneva and was conducted in accordance with its rules and regulations.

Experimental design

Each subject spent 24 h in our respiratory chamber on 3 separate occasions and was randomly assigned to receive 1 of the following 3 treatments orally (in capsular form) 3 time/d (ie, 2 capsules with breakfast, lunch, and dinner): *1*) a green tea extract containing 50 mg caffeine and 90 mg epigallocatechin gallate, *2*) 50 mg caffeine, or *3*) a placebo that consisted of cellulose as inert filler. The dosages represented the amount of caffeine and epigallocatechin gallate (the quantitatively most important catechin polyphenol) in 2 capsules. The green tea extract (code name: AR25) was obtained by alcohol extraction from dry tea leaves of unfermented *Camellia sinensis*, standardized at 25% catechins, and commercially prepared in capsular form under the name Exolise (Arkopharma Laboratories, Nice, France). Note that apart from (−)-epigallocatechin gallate, the green tea extract also contains substantial amounts of other catechins: (−)-epigallocatechin, (−)-epicatechin, and (−)-epicatechin gallate. (−)-Epigallocatechin gallate constitutes ≥50% of the total amount of tea catechins and is believed to be the most pharmacologically active tea catechin (14). In the present study, (−)-epigallocatechin gallate was found to constitute ≈72% of total catechins, such that the amount of total catechins consumed with each meal was 125 mg. Consequently, ingestion of capsules containing the green tea extract AR25 provided daily a total of 150 mg caffeine and 375 mg catechins, of which 270 mg was epigallocatechin gallate. The various treatments in the respiratory chamber were administered in a double-blind design and with a 5–10-d interval between successive 24-h trials for each subject. During the entire study period (lasting 5–6 wk), the subjects were prescribed a weight-maintenance diet consisting of ≈13% of energy as protein, ≈40% as fat, and ≈47% as carbohydrates. During each respiratory chamber trial, this diet was considered the "basal diet," which was fed at an energy level of 1.4 times the estimated basal energy requirements of the subject, predicted from the regression equation of Cunningham (15). Thus, during each of the subject's 3 respiratory chamber trials, the following conditions were the same: energy intake, nutrient composition of the diet, sedentary lifestyle pattern (reading, listening to radio, watching television, etc), pattern of physical activity, meal pattern, and time period for sleeping. No methylxanthine-containing foods or beverages were consumed 24 h before or during the stay in the respiratory chamber. During the first 8 h of each trial, the heart rate was monitored with a portable frequency meter.

Determination of daily energy expenditure and substrate oxidation

EE was continuously monitored by indirect calorimetry during the stay in the respiratory chamber, the details of which were described previously (16). The respiratory chamber had a large window overlooking the streets and was large enough (3 m long × 2.5 m wide × 2.5 m high) to provide the comforts of a hotel. It was furnished with a bed, resting armchair, table, wash basin and water tap, dry toilet, audiovisual equipment (television, video cassette recorder, radio, and tape recorder), intercom, and a telephone. The door was fitted with a double window as well as an air-lock system through which food and other items were provided. Complete privacy was obtainable by pulling a curtain over the windows. The chamber was sufficiently airtight to ensure that air left only through the apparatus that measures its flow rate and gas concentrations. A pump removed air continuously from the chamber at a rate that could be varied from between 50 and 100 L/min, which passed through a mass flow meter for continuous measurement of the flow rate. The effect of pumping air out resulted in air entering the chamber through a special inlet placed in the wall opposite the location where the air left. A fan ensured that the air was mixed inside the chamber and a thermostat ensured the maintenance of a constant and comfortable temperature. Air samples entering and leaving the chamber passed through differential analyzers for continuous

measurements of differences in oxygen and carbon dioxide contents between extracted air and inlet air. These data were continuously fed into an online computerized data acquisition system, from which EE and the respiratory quotient (RQ) were calculated throughout the measurement periods. Measurements were accurate within 1–2%, as described previously (16). The oxidation rates of protein, carbohydrate, and fat were calculated from 24-h EE, RQ, and urinary nitrogen excretion for each 24-h stay in the respiratory chamber (17).

Measurement of urinary nitrogen and catecholamines

During each subject's stay in the respirometer, urine was collected into 2 or more 2-L opaque glass containers (containing 10 mL of 5 mol HCl/L each) over 2 periods to reflect diurnal and nocturnal phases, with the time intervals indicated below. After the 24-h collection period was complete, all urine samples were stored at −20°C until assayed for nitrogen with an autoanalyzer by the method of Kjeldahl and for epinephrine, norepinephrine, and dopamine concentrations by liquid chromatography with electrochemical detection.

Data presentation

EE, RQ, substrate oxidation, and urinary catecholamine data are reported as diurnal (corresponding to the first 15 h in the respiratory chamber, from 0800 to 2300), nocturnal (from 2300 to 0800 the next morning), and total 24-h values.

Statistics

Repeated-measures analysis of variance was used to determine significance. When statistically significant differences were detected, a post hoc pairwise comparison across treatments was performed by using Tukey's test. Significance was set at a P value < 0.05. The statistical analyses were performed by using the computer software program STATISTIK 4.0 (Analytic Software, St Paul).

RESULTS

Energy expenditure

Mean (±SEM) diurnal, nocturnal, and total 24-h EE values are presented in **Table 1**. Significant differences across treatments were observed only for diurnal and total 24-h EE. Diurnal EE was higher during treatment with the green tea extract than during treatment with placebo or caffeine, by 4.5% and 3.2%, respectively, but significantly so only for the green tea extract. Total 24-h EE with the green tea extract, however, was significantly higher than that with both the placebo and caffeine, by 3.5% and 2.8%, respectively. There were no significant differences in diurnal, nocturnal, or total 24-h EE between the caffeine and placebo groups. Individual changes (relative to placebo) in total 24-h EE indicated an increase in only 2 subjects after caffeine treatment, but an increase in 6 of the 10 subjects after treatment with the green tea extract, ranging from 266 to 836 kJ (mean or median of ≈330 kJ). No correlation was observed between the magnitude of thermogenic response and the degree of fatness (BMI or percentage of body fat) of the subjects.

Respiratory quotient and substrate oxidation

RQs are shown in **Table 2**. Significant differences across treatments were found during the diurnal, nocturnal, and total 24-h periods. Treatment with the green tea extract yielded significantly lower values than did the other 2 treatments during all 3 periods. Individual changes indicated that the RQ in most of the subjects (8 of 10) was substantially lower (differences >0.01) after the green tea extract than after the placebo; in 4 of these subjects the difference was ≥0.04. However, no correlation was observed between the magnitude of reduction in the RQ and the degree of fatness (BMI or percentage of body fat) of the subjects.

Because urinary nitrogen losses (and hence protein oxidation) indicated no significant differences across treatments for all 3 periods, the lower RQ during treatment with the green tea extract was due to a shift in substrate utilization in favor of fat oxidation. As indicated in **Table 3**, carbohydrate oxidation was significantly lower ($P < 0.01$) and fat oxidation was significantly higher ($P < 0.001$) after the green tea extract than after the placebo. By contrast, there were no significant differences in substrate oxidation between the caffeine and placebo groups. The relative contribution of protein, carbohydrate, and fat oxidation to daily EE are also presented in Table 3. The contribution of fat oxidation to 24-h EE during treatment with the green tea extract (41.5%) was significantly higher ($P < 0.001$) than during placebo treatment (31.6%).

TABLE 1
Energy expenditure (EE) during diurnal, nocturnal, and total 24-h periods[1]

	Placebo	Caffeine	Green tea	P[2]
		kJ		
Diurnal EE	6463 ± 386	6547 ± 383	6754 ± 352[3]	<0.01
Nocturnal EE	3075 ± 149	3053 ± 148	3112 ± 140	NS
Total 24-h EE	9538 ± 521	9599 ± 518	9867 ± 488[3,4]	<0.01

[1] $\bar{x}$ ± SEM; $n = 10$.
[2] For differences across treatments (ANOVA).
[3] Significantly different from placebo, $P < 0.05$ (post hoc pairwise comparison with Tukey's test).
[4] Significantly different from caffeine, $P < 0.05$ (post hoc pairwise comparison with Tukey's test).

Urinary excretion of catecholamines

Urinary excretion values of catecholamines during the study are shown in **Table 4**. Urinary epinephrine and dopamine were not significantly different across treatments in any of the 3 periods. Urinary norepinephrine and its precursor dopamine tended to be highest during treatment with the green tea extract, although differences across treatments were only significant for total 24-h norepinephrine.

Heart rate

None of the subjects reported any side effects and no significant differences in heart rates across treatments were observed during the first 8 h that the subjects were assessed in the respiratory chamber.

DISCUSSION

Although both coffee and tea are widely consumed worldwide, our knowledge of their influence on energy metabolism has been limited to studies of coffee or to its main pharmacologically active ingredient caffeine. Therefore, the results of the

TABLE 2
Respiratory quotient (RQ) during diurnal, nocturnal and total 24-h periods[1]

	Placebo	Caffeine	Green tea	P^2
Diurnal RQ	0.887 ± 0.0081	0.878 ± 0.0071	0.858 ± 0.009[3]	<0.002
Nocturnal RQ	0.870 ± 0.009	0.864 ± 0.008	0.841 ± 0.01[3]	<0.01
Total 24-h RQ	0.881 ± 0.008	0.873 ± 0.007	0.852 ± 0.009[3]	<0.001

[1] $\bar{x}$ ± SEM; n = 10.
[2] For differences across treatments (ANOVA).
[3] Significantly different from placebo and caffeine, $P < 0.05$ (post hoc pairwise comparison with Tukey's test).

present investigation are the first to show in humans that tea (albeit green tea) also has the potential to influence EE and substrate utilization. Because dietary energy intake and diet composition were identical during all treatments and because the subjects maintained the same feeding and physical activity patterns during each 24-h respiratory chamber trial, the 4% increase in 24-h EE during treatment with the green tea extract essentially reflects its stimulatory effect on thermogenesis. Furthermore, despite the absence of differences in urinary nitrogen excretion, and hence in protein oxidation rates, the observed reductions in RQ during treatment with the green tea extract suggest that fat oxidation was higher and carbohydrate oxidation was lower during this period than during the placebo period. Indeed, calculations of the relative contribution of substrate oxidation to daily EE indicated that the contribution of fat oxidation to 24-h EE, which was 31.6% with the placebo, was higher (41.5%) with the green tea extract. Of particular interest in this study was that the effects of the green tea extract in enhancing thermogenesis and fat oxidation could not be explained solely on the basis of its caffeine content because treatment with an amount of caffeine equivalent to that in the extract failed to alter EE, RQ, or substrate oxidation. The implication of this finding is that these metabolic effects resulted from ingredients other than caffeine in the green tea extract. The most likely explanation for the lack of a thermogenic effect of caffeine is that the dosage (50 mg 3 times/d) was below the threshold for stimulating thermogenesis. On the basis of data from the literature, a single oral dose of ≥100 mg caffeine is required to produce a thermogenic response sustainable for ≥1–2 h, and a stimulatory effect of caffeine per se on 24-h EE under respiratory chamber conditions has only been reported with dosages of 600–1000 mg caffeine/d (18, 19). It is therefore not surprising that in the present study, the administration of caffeine alone (<100 mg with each meal) failed to increase daily EE. Nonetheless, the amount of caffeine consumed during treatment with the green tea extract may have reached the critical dose, which, although ineffective by itself, may have enabled a synergistic interaction with other bioactive ingredients in the green tea extract to promote catecholamine-induced thermogenesis and fat oxidation.

TABLE 3
Substrate oxidation during 24 h in the respiratory chamber[1]

	Placebo	Caffeine	Green tea	P^2
Protein				
(g)	65.6 ± 3.1	66.9 ± 4.7	68.3 ± 3.5	NS
(% of 24-h EE)	13.2 ± 1	13.4 ± 0.98	13.3 ± 0.98	NS
Carbohydrate				
(g)	336 ± 16	324 ± 16	285 ± 17[3]	<0.001
(% of 24-h EE)	55.1 ± 2.4	52.7 ± 2.1	45.2 ± 2.7[4]	<0.001
Fat				
(g)	76.2 ± 10.6	81.9 ± 8.7	103 ± 13[4]	<0.001
(% of 24-h EE)	31.6 ± 3.1	33.8 ± 2.4	41.5 ± 3.1[4]	<0.001

[1] $\bar{x}$ ± SEM; n = 10.
[2] For differences across treatments (ANOVA).
[3] Significantly different from placebo, $P < 0.05$ (post hoc pairwise comparison with Tukey's test).
[4] Significantly different from placebo and caffeine, $P < 0.05$ (post hoc pairwise comparison with Tukey's test).

Mechanism of action

Green tea is well known for being particularly rich in flavonoids (14), and several of these polyphenols—particularly the subclass of flavonoids commonly known as tea catechins—have been shown in vitro to inhibit COMT (11), the enzyme that degrades norepinephrine. Given the important role of the SNS and its neurotransmitter norepinephrine in the control of thermogenesis and fat oxidation, it is conceivable that these catechins, by inhibiting COMT, result in an increase in or a more prolonged effect of norepinephrine on thermogenesis and fat metabolism or both. Support for this contention comes from our previously reported in vitro studies on the respiration rate of brown adipose tissue, which indicated that *1*) a green tea extract (rich in catechin polyphenols and to a lesser extent in caffeine) was more potent than were equimolar concentrations of caffeine alone in stimulating the respiration rate of brown adipose tissue (12), *2*) the thermogenic effect of a green tea extract was markedly potentiated by enhancing the release of norepinephrine from the sympathetic nerve terminals with the use of ephedrine (12), and *3*) the thermogenic effect of a green tea extract could be mimicked by epigallocatechin gallate (20). Furthermore, the assay of urinary catecholamines in the present study of humans showed a tendency for urinary norepinephrine (and its precursor dopamine), but not for epinephrine, to be higher in most subjects during treatment with the green tea extract; however, differences across treatments were only significant for total 24-h norepinephrine excretion. This observation is consistent with the inhibiting effect of green tea on COMT, the consequential reduction in norepinephrine degradation, and hence, the spillover of norepinephrine into the circulation, thereby accounting for the higher urinary excretion of norepinephrine. Such effects, resulting in a prolonged life of norepinephrine in the sympathetic synaptic cleft, could explain the observed effects of the extract in stimulating thermogenesis and fat oxidation.

It can be argued, however, that other tea flavonoids—such as quercetin and myricetin, which have also been shown to inhibit COMT in vitro (11)—may also have played a role in the metabolic effects of the green extract observed in the present study. However, there are only minute amounts of these flavonoids in green tea and their absorption when taken orally is doubtful, par-

TABLE 4
Urinary catecholamines during diurnal, nocturnal, and total 24-h periods[1]

	Placebo	Caffeine	Green tea	P^2
		nmol		
Diurnal				
Epinephrine	66 ± 16	49 ± 4	55 ± 7[3]	NS
Norepinephrine	106 ± 15	127 ± 24	146 ± 23[3]	NS
Dopamine	893 ± 173	946 ± 160	1086 ± 179[3]	NS
Nocturnal				
Epinephrine	12 ± 4	19 ± 4	15 ± 3[3]	NS
Norepinephrine	54 ± 5	61 ± 11	73 ± 7[3]	NS
Dopamine	694 ± 80	632 ± 126	803 ± 105[3]	NS
Total 24 h				
Epinephrine	78 ± 13	67 ± 4	70 ± 8[3]	NS
Norepinephrine	160 ± 14	187 ± 29	219 ± 27[3]	<0.05[4]
Dopamine	1587 ± 187	1578 ± 165	1889 ± 241[3]	NS

[1] $\bar{x}$ ± SEM; $n = 10$.
[2] For differences across treatments (ANOVA).
[3] Significantly different from placebo, $P < 0.05$ (post hoc pairwise comparison with Tukey's test).
[4] $F = 3.96$.

ticularly because of evidence that flavonoids in food cannot generally be absorbed from the small intestine because they are bound to sugars as glycosides. By contrast, catechins are not only present in large quantities in green tea, but they are known to be better absorbed than are flavonoids. Indeed, substantial amounts of epigallocatechin gallate, epigallocatechin, and epicatechin have been measured in the plasma of human volunteers after ingestion of green tea powder, with peak plasma concentrations of catechins (nonconjugated) after 3 h of 3–3% of the ingested dose (21, 22). It is not known whether the relatively low ratios of circulating catechins to ingested catechins can be attributed to an efficient metabolism or to uptake by other tissues. However, the tissue concentrations of at least one of these tea catechins must have been high enough in our study to exert biological effects, as indicated by the stimulatory effect of the green tea extract on energy metabolism. Taken together, the results of these in vitro studies of rat brown adipose tissue thermogenesis (12) and in vivo studies of tea catechin bioavailability in humans (21, 22) suggest that the thermogenic effects of the green tea extract result, at least in part, from interactions between tea catechins, caffeine, and norepinephrine. The proposed mechanism is as follows: the catechins, by inhibiting COMT (and hence prolonging the life of norepinephrine in the synaptic cleft), and caffeine, by inhibiting phosphodiesterases (and hence prolonging the life of cAMP in the cell), result in an increase and more sustained effect of norepinephrine on thermogenesis.

Implications for weight control

First, the effect of the green tea extract on the metabolic rate represents an increase in 24-h EE of ≈4%. It is likely that a major component of this increase in daily EE was due to a cumulative increase in postprandial thermogenesis during consumption of the 3 meals in the diurnal period, particularly because no significant differences in nocturnal EE were observed. If, as generally accepted, thermogenesis contributes 8–10% of daily EE in a typical sedentary man (760–950 kJ in our subjects), this 4% increase in 24-h EE (328 kJ) due to the green tea extract would extrapolate to a 35–43% increase in the thermogenesis compartment of daily EE. This thermogenic effect of the extract (an increase of 328 kJ/d) was comparable with increases in daily EE seen in previous studies with much higher doses of caffeine in postobese and lean subjects (increases of 400 kJ) (18); however, only half of the thermogenic stimulation was the result of a combination of ephedrine and caffeine (800 kJ) (23). The results of these studies together with the results of our in vitro studies in brown adipose tissue—which indicate that the stimulatory effect of the extract on tissue thermogenesis was markedly potentiated in the presence of ephedrine (12, 20)—raise the possibility that the effect of the green tea extract could be greater under conditions of elevated sympathetic tone and norepinephrine release (ie, higher activity of COMT), such as during concomitant treatment with drugs that enhance norepinephrine release or when activity levels are higher than those under the confined and sedentary conditions of a respiratory chamber. Second, the differences in substrate utilization in favor of fat oxidation (lower RQ) in response to the green tea extract were much more consistent than were the differences in EE because lower RQs with the extract than with the placebo were observed in most of the subjects, including in those subjects who did not show a higher EE. This finding with the green tea extract is even more remarkable when compared with data indicating that caffeine ingestion alone, even at doses as high as 1000 mg/d, had no significant effect on the RQ during the diurnal or nocturnal period (19). Third, stimulation of thermogenesis and fat oxidation by the green tea extract was not accompanied by an increase in heart rate. In this respect, the green tea extract is distinct from sympathomimetic drugs, whose use as antiobesity thermogenic agents is limited by their adverse cardiovascular effects and, hence, are particularly inappropriate for obese individuals with hypertension and other cardiovascular complications.

Conclusion

In conclusion, oral administration of the green tea extract stimulated thermogenesis and fat oxidation and thus has the potential to influence body weight and body composition via changes in both EE and substrate utilization.

REFERENCES

1. Landsberg L, Young JB. Sympathoadrenal activity and obesity: physiological rationale for the use of adrenergic thermogenic drugs. Int J Obes Relat Metab Disord 1993;65:S29–34.
2. Dulloo AG. Strategies to counteract readjustments towards lower metabolic rates during obesity management. Nutrition 1993;9:366–72.
3. Arch JRS, Wilson S. Prospects for β_3-adrenoceptor agonists in the treatment of obesity and diabetes. Int J Obes Relat Metab Disord 1996;20:191–9.
4. Dulloo AG. Spicing fat for combustion. Br J Nutr 1998;80:493–4.
5. Henry CJK, Emery B. Effects of spiced food on metabolic rate. Hum Nutr Clin Nutr 1986;40C:165–8.
6. Yoshioka M, St-Pierre S, Suzuki M, Tremblay A. Effects of red pepper added to high-fat and high-carbohydrate meals on energy metabolism and substrate utilization in Japanese women. Br J Nutr 1998;80:503–10.
7. Dulloo AG. Ephedrine, xanthines and prostaglandin-inhibitors: actions and interactions in the stimulation of thermogenesis. Int J Obes Relat Metab Disord 1993;17:S35–40.
8. Toubro S, Astrup A, Breum L, Quaade F. Safety and efficacy of long-term treatment with ephedrine, caffeine and an ephedrine/caffeine mixture. Int J Obes Relat Metab Disord 1993;17:S69–72.

9. Dulloo AG, Seydoux J, Girardier L. Potentiation of the thermogenic antiobesity effects of ephedrine by dietary methylxanthines: adenosine antagonism or phosphodiesterase inhibition? Metabolism 1992;41:1233–41.
10. Durand J, Giacobino JP, Girardier L. Catechol-*O*-methyl-transferase activity in whole brown adipose tissue of rat in vitro. In: Girardier L, Seydoux J, eds. Effectors of thermogenesis. Basel, Switzerland: Birkhauser, 1977:45–53.
11. Borchardt RT, Huber JA. Catechol-*O*-methyltransferase: structure-activity relationships for inhibition by flavonoids. J Med Chem 1975;18:120–2.
12. Dulloo AG, Seydoux J, Girardier L. Tealine and thermogenesis: interactions between polyphenols, caffeine and sympathetic activity. Int J Obes Relat Metab Disord 1996;20(suppl):71(abstr).
13. Durnin JVGA, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness measurements of 481 men and women aged 16–72 years. Br J Nutr 1974;32:77–97.
14. Stagg GV, Millin DJ. The nutritional and therapeutic value of tea—a review. J Sci Food Agric 1975;26:1439–59.
15. Cunningham JJ. Body composition as a determinant of energy expenditure: a synthetic review and a proposed general prediction equation. Am J Clin Nutr 1991;54:963–9.
16. Dulloo AG, Fathi M, Mensi N, Girardier L. Twenty-four hour energy expenditure and urinary catecholamines of humans consuming low-to-moderate amounts of medium-chain-triglycerides: a dose-response study in a respiratory chamber. Eur J Clin Nutr 1996;50:152–8.
17. Jequier E, Acheson KJ, Schutz Y. Assessment of energy expenditure and fuel utilization in man. Annu Rev Nutr 1987;7:187–208.
18. Dulloo AG, Geissler CA, Horton T, Collins A, Miller DS. Normal caffeine consumption: influence on thermogenesis and daily energy expenditure in lean and post-obese human volunteers. Am J Clin Nutr 1989;49:44–50.
19. Bracco D, Ferrarra JM, Arnaud MJ, Jéquier E, Schutz Y. Effects of caffeine on energy metabolism, heart rate, and methylxanthine metabolism in lean and obese women. Am J Physiol 1995;269:E671–8.
20. Dulloo AG, Seydoux J, Girardier L, Chantra P, Vandermander J. Green tea and thermogenesis: interactions between catechin-polyphenols, caffeine and sympathetic activity. Int J Obes Relat Metab Disord (in press).
21. Lee MJ, Wang ZY, Li H, et al. Analysis of plasma and urinary tea polyphenols in human subjects. Cancer Epidemiol Biomarkers Prev 1995;44:393–9.
22. Hollman PCH, Tijburg LBM, Yang CS. Bioavailability of flavonoids from tea. Crit Rev Food Sci Nutr 1997;37:719–38.
23. Dulloo AG, Miller DS. The thermogenic properties of ephedrine/methylxanthine mixtures: human studies. Int J Obes 1986;10:467–81.

Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans[1–3]

Abdul G Dulloo, Claudette Duret, Dorothée Rohrer, Lucien Girardier, Nouri Mensi, Marc Fathi, Philippe Chantre, and Jacques Vandermander

ABSTRACT

Background: Current interest in the role of functional foods in weight control has focused on plant ingredients capable of interfering with the sympathoadrenal system.

Objective: We investigated whether a green tea extract, by virtue of its high content of caffeine and catechin polyphenols, could increase 24-h energy expenditure (EE) and fat oxidation in humans.

Design: Twenty-four–hour EE, the respiratory quotient (RQ), and the urinary excretion of nitrogen and catecholamines were measured in a respiratory chamber in 10 healthy men. On 3 separate occasions, subjects were randomly assigned among 3 treatments: green tea extract (50 mg caffeine and 90 mg epigallocatechin gallate), caffeine (50 mg), and placebo, which they ingested at breakfast, lunch, and dinner.

Results: Relative to placebo, treatment with the green tea extract resulted in a significant increase in 24-h EE (4%; $P < 0.01$) and a significant decrease in 24-h RQ (from 0.88 to 0.85; $P < 0.001$) without any change in urinary nitrogen. Twenty-four–hour urinary norepinephrine excretion was higher during treatment with the green tea extract than with the placebo (40%, $P < 0.05$). Treatment with caffeine in amounts equivalent to those found in the green tea extract had no effect on EE and RQ nor on urinary nitrogen or catecholamines.

Conclusions: Green tea has thermogenic properties and promotes fat oxidation beyond that explained by its caffeine content per se. The green tea extract may play a role in the control of body composition via sympathetic activation of thermogenesis, fat oxidation, or both. *Am J Clin Nutr* 1999;70:1040–5.

KEY WORDS Obesity, thermogenesis, catechins, polyphenols, caffeine, sympathetic nervous system, green tea, fat oxidation, catecholamines, men

INTRODUCTION

Fundamentally, there are only 2 ways to treat obesity: reduce energy intake or increase energy expenditure (EE). Because thermogenesis and fat oxidation are to a large extent under the control of the sympathetic nervous system (SNS), approaches that mimic or interfere with the SNS and its neurotransmitter norepinephrine offer a rational approach for obesity management (1–3). In this context, there has been renewed interest in the potential thermogenic effects of many compounds extracted from plants (eg, caffeine from coffee and tea, ephedrine from ephedra, and capsaicin from pungent spices), largely because of their potential to modulate catecholamine release and activity (4) . For example, capsaicin-rich foods (eg, chili peppers and red peppers) have been shown to stimulate fat oxidation and thermogenesis in humans (5, 6), and caffeine in relatively small amounts can potentiate thermogenesis induced by sympathetic stimuli, whether in response to cold, moderate exercise, or sympathomimetic drugs like ephedrine (7). In fact, long-term clinical trials have shown greater losses in body weight and body fat in obese patients treated with a combination of caffeine and ephedrine than in those treated with placebo, caffeine, or ephedrine alone (8).

Previous work in our laboratory, in which an in vitro system was used to measure the respiration rate of brown adipose tissue in rats, suggests that the interaction between caffeine and ephedrine resides in ephedrine's induced enhancement of sympathetic neural release of norepinephrine together with caffeine's ability to inhibit the phosphodiesterase-induced degradation of intracellular cyclic AMP (cAMP), and, to a lesser extent, caffeine's antagonism of the negative modulatory effect of adenosine on increased norepinephrine release (9). The net result, therefore, would be an elevated cellular concentration of cAMP—a critical intracellular mediator for the actions of catecholamines on thermogenesis. Apart from phosphodiesterases, adenosine, and certain prostaglandins, the concentration of norepinephrine at the synaptic junction and its interaction with adrenoceptors is also likely to be negatively modulated through its enzymatic degradation, namely by catechol *O*-methyltransferase (COMT) (10). Given

[1] From the Department of Physiology, Faculty of Medicine, University of Geneva; Geneva University Hospital; and Laboratoires Arkopharma, Nice, France.

[2] Supported in part by Arkopharma Laboratories and by the Swiss National Science Research Fund.

[3] Address reprint requests to AG Dulloo, Institute of Physiology, University of Fribourg, Rue de Musée 5, CH-1700 Fribourg, Switzerland. E-mail: abdul.dulloo@.unifr.ch.

Received December 16, 1998.

Accepted for publication March 31, 1999.

Am J Clin Nutr 1999;70:1040–5. Printed in USA.

evidence that this enzyme can be inhibited by certain tea polyphenols (11), we recently investigated in our in vitro system whether an extract of green tea, by virtue of its high content of both caffeine and catechin polyphenols, could be an effective promoter of thermogenesis. These in vitro results (12) can be summarized as follows: *1*) the green tea extract was found to be more effective than were equivalent amounts of caffeine in stimulating peripheral tissue thermogenesis, and *2*) this difference between the green tea extract and equimolar caffeine in activating thermogenesis was much more marked under conditions of increased norepinephrine release because the synergistic interaction between the green tea extract and ephedrine on tissue thermogenesis was much more pronounced than that of caffeine or ephedrine.

On the basis of these in vitro data, our main objectives in this study were 2-fold: *1*) to examine the extent to which daily administration of capsules containing a green tea extract (containing catechin polyphenols and caffeine in amounts comparable with those commonly consumed in green tea beverages in Asian communities) would stimulate thermogenesis and increase daily EE in humans, and *2*) to determine whether the effects of the green tea extract on the metabolic rate and substrate oxidation in humans would be greater than that explained by its caffeine content per se.

SUBJECTS AND METHODS

Subjects

Healthy young men were recruited from the student and staff population of our University after complete medical and nutritional histories were obtained by use of a questionnaire. Smokers, competitive athletes, and persons who engaged in intense physical activities or who had a history of weight loss were not eligible for inclusion in the study. Inclusion criteria included body fatnesses ranging from lean to mildly obese (8–30% body fat). All selected subjects habitually consumed a typical Western diet, with fat contributing 35–40% of dietary energy intake, and their estimated intake of methylxanthines (mostly as caffeine-containing beverages) ranged from 100 to 200 mg/d. At the onset of the study, body weight and height were measured and body fat was determined by the method of Durnin and Womersley (13) from measurements of skinfold thicknesses taken at 4 sites with a Harpenden skinfold caliper (British Indicators, Ltd, London); fat-free-mass (FFM) was calculated as the difference between body weight and body fat. Mean (±SEM) values for some of the physical characteristics of the 10 men participating in this study were as follows: age, 25 ± 1 y; height, 177 ± 3 cm; weight, 78.7 ± 4.3 kg; body mass index (BMI; in kg/m^2), 25.1 ± 1.2; percentage body fat, 18.2 ± 1.8%; and FFM, 63.8 ± 2.5 kg. The study was approved by the Ethical Committee for Human Experimentation of the University of Geneva and was conducted in accordance with its rules and regulations.

Experimental design

Each subject spent 24 h in our respiratory chamber on 3 separate occasions and was randomly assigned to receive 1 of the following 3 treatments orally (in capsular form) 3 time/d (ie, 2 capsules with breakfast, lunch, and dinner): *1*) a green tea extract containing 50 mg caffeine and 90 mg epigallocatechin gallate, *2*) 50 mg caffeine, or *3*) a placebo that consisted of cellulose as inert filler. The dosages represented the amount of caffeine and epigallocatechin gallate (the quantitatively most important catechin polyphenol) in 2 capsules. The green tea extract (code name: AR25) was obtained by alcohol extraction from dry tea leaves of unfermented *Camellia sinensis*, standardized at 25% catechins, and commercially prepared in capsular form under the name Exolise (Arkopharma Laboratories, Nice, France). Note that apart from (−)-epigallocatechin gallate, the green tea extract also contains substantial amounts of other catechins: (−)-epigallocatechin, (−)-epicatechin, and (−)-epicatechin gallate. (−)-Epigallocatechin gallate constitutes ≥50% of the total amount of tea catechins and is believed to be the most pharmacologically active tea catechin (14). In the present study, (−)-epigallocatechin gallate was found to constitute ≈72% of total catechins, such that the amount of total catechins consumed with each meal was 125 mg. Consequently, ingestion of capsules containing the green tea extract AR25 provided daily a total of 150 mg caffeine and 375 mg catechins, of which 270 mg was epigallocatechin gallate. The various treatments in the respiratory chamber were administered in a double-blind design and with a 5–10-d interval between successive 24-h trials for each subject. During the entire study period (lasting 5–6 wk), the subjects were prescribed a weight-maintenance diet consisting of ≈13% of energy as protein, ≈40% as fat, and ≈47% as carbohydrates. During each respiratory chamber trial, this diet was considered the "basal diet," which was fed at an energy level of 1.4 times the estimated basal energy requirements of the subject, predicted from the regression equation of Cunningham (15). Thus, during each of the subject's 3 respiratory chamber trials, the following conditions were the same: energy intake, nutrient composition of the diet, sedentary lifestyle pattern (reading, listening to radio, watching television, etc), pattern of physical activity, meal pattern, and time period for sleeping. No methylxanthine-containing foods or beverages were consumed 24 h before or during the stay in the respiratory chamber. During the first 8 h of each trial, the heart rate was monitored with a portable frequency meter.

Determination of daily energy expenditure and substrate oxidation

EE was continuously monitored by indirect calorimetry during the stay in the respiratory chamber, the details of which were described previously (16). The respiratory chamber had a large window overlooking the streets and was large enough (3 m long × 2.5 m wide × 2.5 m high) to provide the comforts of a hotel. It was furnished with a bed, resting armchair, table, wash basin and water tap, dry toilet, audiovisual equipment (television, video cassette recorder, radio, and tape recorder), intercom, and a telephone. The door was fitted with a double window as well as an air-lock system through which food and other items were provided. Complete privacy was obtainable by pulling a curtain over the windows. The chamber was sufficiently airtight to ensure that air left only through the apparatus that measures its flow rate and gas concentrations. A pump removed air continuously from the chamber at a rate that could be varied from between 50 and 100 L/min, which passed through a mass flow meter for continuous measurement of the flow rate. The effect of pumping air out resulted in air entering the chamber through a special inlet placed in the wall opposite the location where the air left. A fan ensured that the air was mixed inside the chamber and a thermostat ensured the maintenance of a constant and comfortable temperature. Air samples entering and leaving the chamber passed through differential analyzers for continuous

measurements of differences in oxygen and carbon dioxide contents between extracted air and inlet air. These data were continuously fed into an online computerized data acquisition system, from which EE and the respiratory quotient (RQ) were calculated throughout the measurement periods. Measurements were accurate within 1–2%, as described previously (16). The oxidation rates of protein, carbohydrate, and fat were calculated from 24-h EE, RQ, and urinary nitrogen excretion for each 24-h stay in the respiratory chamber (17).

Measurement of urinary nitrogen and catecholamines

During each subject's stay in the respirometer, urine was collected into 2 or more 2-L opaque glass containers (containing 10 mL of 5 mol HCl/L each) over 2 periods to reflect diurnal and nocturnal phases, with the time intervals indicated below. After the 24-h collection period was complete, all urine samples were stored at −20°C until assayed for nitrogen with an autoanalyzer by the method of Kjeldahl and for epinephrine, norepinephrine, and dopamine concentrations by liquid chromatography with electrochemical detection.

Data presentation

EE, RQ, substrate oxidation, and urinary catecholamine data are reported as diurnal (corresponding to the first 15 h in the respiratory chamber, from 0800 to 2300), nocturnal (from 2300 to 0800 the next morning), and total 24-h values.

Statistics

Repeated-measures analysis of variance was used to determine significance. When statistically significant differences were detected, a post hoc pairwise comparison across treatments was performed by using Tukey's test. Significance was set at a P value < 0.05. The statistical analyses were performed by using the computer software program STATISTIK 4.0 (Analytic Software, St Paul).

RESULTS

Energy expenditure

Mean (±SEM) diurnal, nocturnal, and total 24-h EE values are presented in **Table 1**. Significant differences across treatments were observed only for diurnal and total 24-h EE. Diurnal EE was higher during treatment with the green tea extract than during treatment with placebo or caffeine, by 4.5% and 3.2%, respectively, but significantly so only for the green tea extract. Total 24-h EE with the green tea extract, however, was significantly higher than that with both the placebo and caffeine, by 3.5% and 2.8%, respectively. There were no significant differences in diurnal, nocturnal, or total 24-h EE between the caffeine and placebo groups. Individual changes (relative to placebo) in total 24-h EE indicated an increase in only 2 subjects after caffeine treatment, but an increase in 6 of the 10 subjects after treatment with the green tea extract, ranging from 266 to 836 kJ (mean or median of ≈330 kJ). No correlation was observed between the magnitude of thermogenic response and the degree of fatness (BMI or percentage of body fat) of the subjects.

Respiratory quotient and substrate oxidation

RQs are shown in **Table 2**. Significant differences across treatments were found during the diurnal, nocturnal, and total 24-h periods. Treatment with the green tea extract yielded significantly lower values than did the other 2 treatments during all 3 periods. Individual changes indicated that the RQ in most of the subjects (8 of 10) was substantially lower (differences >0.01) after the green tea extract than after the placebo; in 4 of these subjects the difference was ≥0.04. However, no correlation was observed between the magnitude of reduction in the RQ and the degree of fatness (BMI or percentage of body fat) of the subjects.

Because urinary nitrogen losses (and hence protein oxidation) indicated no significant differences across treatments for all 3 periods, the lower RQ during treatment with the green tea extract was due to a shift in substrate utilization in favor of fat oxidation. As indicated in **Table 3**, carbohydrate oxidation was significantly lower ($P < 0.01$) and fat oxidation was significantly higher ($P < 0.001$) after the green tea extract than after the placebo. By contrast, there were no significant differences in substrate oxidation between the caffeine and placebo groups. The relative contribution of protein, carbohydrate, and fat oxidation to daily EE are also presented in Table 3. The contribution of fat oxidation to 24-h EE during treatment with the green tea extract (41.5%) was significantly higher ($P < 0.001$) than during placebo treatment (31.6%).

TABLE 1
Energy expenditure (EE) during diurnal, nocturnal, and total 24-h periods[1]

	Placebo	Caffeine	Green tea	P[2]
		kJ		
Diurnal EE	6463 ± 386	6547 ± 383	6754 ± 352[3]	<0.01
Nocturnal EE	3075 ± 149	3053 ± 148	3112 ± 140	NS
Total 24-h EE	9538 ± 521	9599 ± 518	9867 ± 488[3,4]	<0.01

[1] $\bar{x}$ ± SEM; $n = 10$.
[2] For differences across treatments (ANOVA).
[3] Significantly different from placebo, $P < 0.05$ (post hoc pairwise comparison with Tukey's test).
[4] Significantly different from caffeine, $P < 0.05$ (post hoc pairwise comparison with Tukey's test).

Urinary excretion of catecholamines

Urinary excretion values of catecholamines during the study are shown in **Table 4**. Urinary epinephrine and dopamine were not significantly different across treatments in any of the 3 periods. Urinary norepinephrine and its precursor dopamine tended to be highest during treatment with the green tea extract, although differences across treatments were only significant for total 24-h norepinephrine.

Heart rate

None of the subjects reported any side effects and no significant differences in heart rates across treatments were observed during the first 8 h that the subjects were assessed in the respiratory chamber.

DISCUSSION

Although both coffee and tea are widely consumed worldwide, our knowledge of their influence on energy metabolism has been limited to studies of coffee or to its main pharmacologically active ingredient caffeine. Therefore, the results of the

TABLE 2
Respiratory quotient (RQ) during diurnal, nocturnal and total 24-h periods[1]

	Placebo	Caffeine	Green tea	P^2
Diurnal RQ	0.887 ± 0.0081	0.878 ± 0.0071	0.858 ± 0.009[3]	<0.002
Nocturnal RQ	0.870 ± 0.009	0.864 ± 0.008	0.841 ± 0.01[3]	<0.01
Total 24-h RQ	0.881 ± 0.008	0.873 ± 0.007	0.852 ± 0.009[3]	<0.001

[1] $\bar{x}$ ± SEM; n = 10.
[2] For differences across treatments (ANOVA).
[3] Significantly different from placebo and caffeine, $P < 0.05$ (post hoc pairwise comparison with Tukey's test).

present investigation are the first to show in humans that tea (albeit green tea) also has the potential to influence EE and substrate utilization. Because dietary energy intake and diet composition were identical during all treatments and because the subjects maintained the same feeding and physical activity patterns during each 24-h respiratory chamber trial, the 4% increase in 24-h EE during treatment with the green tea extract essentially reflects its stimulatory effect on thermogenesis. Furthermore, despite the absence of differences in urinary nitrogen excretion, and hence in protein oxidation rates, the observed reductions in RQ during treatment with the green tea extract suggest that fat oxidation was higher and carbohydrate oxidation was lower during this period than during the placebo period. Indeed, calculations of the relative contribution of substrate oxidation to daily EE indicated that the contribution of fat oxidation to 24-h EE, which was 31.6% with the placebo, was higher (41.5%) with the green tea extract. Of particular interest in this study was that the effects of the green tea extract in enhancing thermogenesis and fat oxidation could not be explained solely on the basis of its caffeine content because treatment with an amount of caffeine equivalent to that in the extract failed to alter EE, RQ, or substrate oxidation. The implication of this finding is that these metabolic effects resulted from ingredients other than caffeine in the green tea extract. The most likely explanation for the lack of a thermogenic effect of caffeine is that the dosage (50 mg 3 times/d) was below the threshold for stimulating thermogenesis. On the basis of data from the literature, a single oral dose of ≥100 mg caffeine is required to produce a thermogenic response sustainable for ≥1–2 h, and a stimulatory effect of caffeine per se on 24-h EE under respiratory chamber conditions has only been reported with dosages of 600–1000 mg caffeine/d (18, 19). It is therefore not surprising that in the present study, the administration of caffeine alone (<100 mg with each meal) failed to increase daily EE. Nonetheless, the amount of caffeine consumed during treatment with the green tea extract may have reached the critical dose, which, although ineffective by itself, may have enabled a synergistic interaction with other bioactive ingredients in the green tea extract to promote catecholamine-induced thermogenesis and fat oxidation.

TABLE 3
Substrate oxidation during 24 h in the respiratory chamber[1]

	Placebo	Caffeine	Green tea	P^2
Protein				
(g)	65.6 ± 3.1	66.9 ± 4.7	68.3 ± 3.5	NS
(% of 24-h EE)	13.2 ± 1	13.4 ± 0.98	13.3 ± 0.98	NS
Carbohydrate				
(g)	336 ± 16	324 ± 16	285 ± 17[3]	<0.001
(% of 24-h EE)	55.1 ± 2.4	52.7 ± 2.1	45.2 ± 2.7[4]	<0.001
Fat				
(g)	76.2 ± 10.6	81.9 ± 8.7	103 ± 13[4]	<0.001
(% of 24-h EE)	31.6 ± 3.1	33.8 ± 2.4	41.5 ± 3.1[4]	<0.001

[1] $\bar{x}$ ± SEM; n = 10.
[2] For differences across treatments (ANOVA).
[3] Significantly different from placebo, $P < 0.05$ (post hoc pairwise comparison with Tukey's test).
[4] Significantly different from placebo and caffeine, $P < 0.05$ (post hoc pairwise comparison with Tukey's test).

Mechanism of action

Green tea is well known for being particularly rich in flavonoids (14), and several of these polyphenols—particularly the subclass of flavonoids commonly known as tea catechins—have been shown in vitro to inhibit COMT (11), the enzyme that degrades norepinephrine. Given the important role of the SNS and its neurotransmitter norepinephrine in the control of thermogenesis and fat oxidation, it is conceivable that these catechins, by inhibiting COMT, result in an increase in or a more prolonged effect of norepinephrine on thermogenesis and fat metabolism or both. Support for this contention comes from our previously reported in vitro studies on the respiration rate of brown adipose tissue, which indicated that *1*) a green tea extract (rich in catechin polyphenols and to a lesser extent in caffeine) was more potent than were equimolar concentrations of caffeine alone in stimulating the respiration rate of brown adipose tissue (12), *2*) the thermogenic effect of a green tea extract was markedly potentiated by enhancing the release of norepinephrine from the sympathetic nerve terminals with the use of ephedrine (12), and *3*) the thermogenic effect of a green tea extract could be mimicked by epigallocatechin gallate (20). Furthermore, the assay of urinary catecholamines in the present study of humans showed a tendency for urinary norepinephrine (and its precursor dopamine), but not for epinephrine, to be higher in most subjects during treatment with the green tea extract; however, differences across treatments were only significant for total 24-h norepinephrine excretion. This observation is consistent with the inhibiting effect of green tea on COMT, the consequential reduction in norepinephrine degradation, and hence, the spillover of norepinephrine into the circulation, thereby accounting for the higher urinary excretion of norepinephrine. Such effects, resulting in a prolonged life of norepinephrine in the sympathetic synaptic cleft, could explain the observed effects of the extract in stimulating thermogenesis and fat oxidation.

It can be argued, however, that other tea flavonoids—such as quercetin and myricetin, which have also been shown to inhibit COMT in vitro (11)—may also have played a role in the metabolic effects of the green extract observed in the present study. However, there are only minute amounts of these flavonoids in green tea and their absorption when taken orally is doubtful, par-

TABLE 4
Urinary catecholamines during diurnal, nocturnal, and total 24-h periods[1]

	Placebo	Caffeine	Green tea	P^2
		nmol		
Diurnal				
Epinephrine	66 ± 16	49 ± 4	55 ± 7[3]	NS
Norepinephrine	106 ± 15	127 ± 24	146 ± 23[3]	NS
Dopamine	893 ± 173	946 ± 160	1086 ± 179[3]	NS
Nocturnal				
Epinephrine	12 ± 4	19 ± 4	15 ± 3[3]	NS
Norepinephrine	54 ± 5	61 ± 11	73 ± 7[3]	NS
Dopamine	694 ± 80	632 ± 126	803 ± 105[3]	NS
Total 24 h				
Epinephrine	78 ± 13	67 ± 4	70 ± 8[3]	NS
Norepinephrine	160 ± 14	187 ± 29	219 ± 27[3]	<0.05[4]
Dopamine	1587 ± 187	1578 ± 165	1889 ± 241[3]	NS

[1] $\bar{x}$ ± SEM; $n = 10$.
[2] For differences across treatments (ANOVA).
[3] Significantly different from placebo, $P < 0.05$ (post hoc pairwise comparison with Tukey's test).
[4] $F = 3.96$.

ticularly because of evidence that flavonoids in food cannot generally be absorbed from the small intestine because they are bound to sugars as glycosides. By contrast, catechins are not only present in large quantities in green tea, but they are known to be better absorbed than are flavonoids. Indeed, substantial amounts of epigallocatechin gallate, epigallocatechin, and epicatechin have been measured in the plasma of human volunteers after ingestion of green tea powder, with peak plasma concentrations of catechins (nonconjugated) after 3 h of 3–3% of the ingested dose (21, 22). It is not known whether the relatively low ratios of circulating catechins to ingested catechins can be attributed to an efficient metabolism or to uptake by other tissues. However, the tissue concentrations of at least one of these tea catechins must have been high enough in our study to exert biological effects, as indicated by the stimulatory effect of the green tea extract on energy metabolism. Taken together, the results of these in vitro studies of rat brown adipose tissue thermogenesis (12) and in vivo studies of tea catechin bioavailability in humans (21, 22) suggest that the thermogenic effects of the green tea extract result, at least in part, from interactions between tea catechins, caffeine, and norepinephrine. The proposed mechanism is as follows: the catechins, by inhibiting COMT (and hence prolonging the life of norepinephrine in the synaptic cleft), and caffeine, by inhibiting phosphodiesterases (and hence prolonging the life of cAMP in the cell), result in an increase and more sustained effect of norepinephrine on thermogenesis.

Implications for weight control

First, the effect of the green tea extract on the metabolic rate represents an increase in 24-h EE of ≈4%. It is likely that a major component of this increase in daily EE was due to a cumulative increase in postprandial thermogenesis during consumption of the 3 meals in the diurnal period, particularly because no significant differences in nocturnal EE were observed. If, as generally accepted, thermogenesis contributes 8–10% of daily EE in a typical sedentary man (760–950 kJ in our subjects), this 4% increase in 24-h EE (328 kJ) due to the green tea extract would extrapolate to a 35–43% increase in the thermogenesis compartment of daily EE. This thermogenic effect of the extract (an increase of 328 kJ/d) was comparable with increases in daily EE seen in previous studies with much higher doses of caffeine in postobese and lean subjects (increases of 400 kJ) (18); however, only half of the thermogenic stimulation was the result of a combination of ephedrine and caffeine (800 kJ) (23). The results of these studies together with the results of our in vitro studies in brown adipose tissue—which indicate that the stimulatory effect of the extract on tissue thermogenesis was markedly potentiated in the presence of ephedrine (12, 20)—raise the possibility that the effect of the green tea extract could be greater under conditions of elevated sympathetic tone and norepinephrine release (ie, higher activity of COMT), such as during concomitant treatment with drugs that enhance norepinephrine release or when activity levels are higher than those under the confined and sedentary conditions of a respiratory chamber. Second, the differences in substrate utilization in favor of fat oxidation (lower RQ) in response to the green tea extract were much more consistent than were the differences in EE because lower RQs with the extract than with the placebo were observed in most of the subjects, including in those subjects who did not show a higher EE. This finding with the green tea extract is even more remarkable when compared with data indicating that caffeine ingestion alone, even at doses as high as 1000 mg/d, had no significant effect on the RQ during the diurnal or nocturnal period (19). Third, stimulation of thermogenesis and fat oxidation by the green tea extract was not accompanied by an increase in heart rate. In this respect, the green tea extract is distinct from sympathomimetic drugs, whose use as antiobesity thermogenic agents is limited by their adverse cardiovascular effects and, hence, are particularly inappropriate for obese individuals with hypertension and other cardiovascular complications.

Conclusion

In conclusion, oral administration of the green tea extract stimulated thermogenesis and fat oxidation and thus has the potential to influence body weight and body composition via changes in both EE and substrate utilization.

REFERENCES

1. Landsberg L, Young JB. Sympathoadrenal activity and obesity: physiological rationale for the use of adrenergic thermogenic drugs. Int J Obes Relat Metab Disord 1993;65:S29–34.
2. Dulloo AG. Strategies to counteract readjustments towards lower metabolic rates during obesity management. Nutrition 1993;9:366–72.
3. Arch JRS, Wilson S. Prospects for β_3-adrenoceptor agonists in the treatment of obesity and diabetes. Int J Obes Relat Metab Disord 1996;20:191–9.
4. Dulloo AG. Spicing fat for combustion. Br J Nutr 1998;80:493–4.
5. Henry CJK, Emery B. Effects of spiced food on metabolic rate. Hum Nutr Clin Nutr 1986;40C:165–8.
6. Yoshioka M, St-Pierre S, Suzuki M, Tremblay A. Effects of red pepper added to high-fat and high-carbohydrate meals on energy metabolism and substrate utilization in Japanese women. Br J Nutr 1998;80:503–10.
7. Dulloo AG. Ephedrine, xanthines and prostaglandin-inhibitors: actions and interactions in the stimulation of thermogenesis. Int J Obes Relat Metab Disord 1993;17:S35–40.
8. Toubro S, Astrup A, Breum L, Quaade F. Safety and efficacy of long-term treatment with ephedrine, caffeine and an ephedrine/caffeine mixture. Int J Obes Relat Metab Disord 1993;17:S69–72.

9. Dulloo AG, Seydoux J, Girardier L. Potentiation of the thermogenic antiobesity effects of ephedrine by dietary methylxanthines: adenosine antagonism or phosphodiesterase inhibition? Metabolism 1992;41:1233–41.
10. Durand J, Giacobino JP, Girardier L. Catechol-*O*-methyl-transferase activity in whole brown adipose tissue of rat in vitro. In: Girardier L, Seydoux J, eds. Effectors of thermogenesis. Basel, Switzerland: Birkhauser, 1977:45–53.
11. Borchardt RT, Huber JA. Catechol-*O*-methyltransferase: structure-activity relationships for inhibition by flavonoids. J Med Chem 1975;18:120–2.
12. Dulloo AG, Seydoux J, Girardier L. Tealine and thermogenesis: interactions between polyphenols, caffeine and sympathetic activity. Int J Obes Relat Metab Disord 1996;20(suppl):71(abstr).
13. Durnin JVGA, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness measurements of 481 men and women aged 16–72 years. Br J Nutr 1974;32:77–97.
14. Stagg GV, Millin DJ. The nutritional and therapeutic value of tea—a review. J Sci Food Agric 1975;26:1439–59.
15. Cunningham JJ. Body composition as a determinant of energy expenditure: a synthetic review and a proposed general prediction equation. Am J Clin Nutr 1991;54:963–9.
16. Dulloo AG, Fathi M, Mensi N, Girardier L. Twenty-four hour energy expenditure and urinary catecholamines of humans consuming low-to-moderate amounts of medium-chain-triglycerides: a dose-response study in a respiratory chamber. Eur J Clin Nutr 1996;50:152–8.
17. Jequier E, Acheson KJ, Schutz Y. Assessment of energy expenditure and fuel utilization in man. Annu Rev Nutr 1987;7:187–208.
18. Dulloo AG, Geissler CA, Horton T, Collins A, Miller DS. Normal caffeine consumption: influence on thermogenesis and daily energy expenditure in lean and post-obese human volunteers. Am J Clin Nutr 1989;49:44–50.
19. Bracco D, Ferrarra JM, Arnaud MJ, Jéquier E, Schutz Y. Effects of caffeine on energy metabolism, heart rate, and methylxanthine metabolism in lean and obese women. Am J Physiol 1995;269:E671–8.
20. Dulloo AG, Seydoux J, Girardier L, Chantra P, Vandermander J. Green tea and thermogenesis: interactions between catechin-polyphenols, caffeine and sympathetic activity. Int J Obes Relat Metab Disord (in press).
21. Lee MJ, Wang ZY, Li H, et al. Analysis of plasma and urinary tea polyphenols in human subjects. Cancer Epidemiol Biomarkers Prev 1995;44:393–9.
22. Hollman PCH, Tijburg LBM, Yang CS. Bioavailability of flavonoids from tea. Crit Rev Food Sci Nutr 1997;37:719–38.
23. Dulloo AG, Miller DS. The thermogenic properties of ephedrine/methylxanthine mixtures: human studies. Int J Obes 1986;10:467–81.

Featured on MedicineNet
- Depression Tips Slideshow
- Manage Type 2 Diabetes
- Myths About Depression

FDA: Stop Using Hydroxycut Related Articles
- Abdominal Pain
- Itch
- Jaundice
- ma-huang (Ephedra sp)-oral
- Nausea and Vomiting
- Obesity (Weight Loss)
- Rhabdomyolysis
- Seizure

New on MedicineNet
- Blood Transfusion FAQs
- Autism Symptoms
- UTI Symptoms and Treatment
- Plant Thorn Arthritis
- Rifaximin (Xifaxan) for IBS
- 10 Men's Health Problems
- Healthy Living Tips

Skin Conditions Gallery
Visual Library

Latest MedicineNet News
- Nurses' Long Shifts May Put Patients at Risk
- Doctors Still Fear Malpractice Lawsuits
- Young Female Chimps Use Sticks as 'Dolls'
- 'Walkable' Communities More Close-Knit
- Accidental Falls a Leading Cause of Head Injury
- Want More News? Sign Up for MedicineNet Newsletters!
- Health News Feed

FONT SIZE
A A A

FDA: Stop Using Hydroxycut

Medical Author: Melissa Conrad Stoppler, MD
Medical Editor: William C. Shiel Jr., MD, FACP, FACR

Hydroxycut weight-loss supplements have been linked to serious medical problems, including one death, according to the United States Food and Drug Administration (FDA) warning issued on May 1, 2009. In all, 23 reports were received by the FDA of serious health problems that developed in people using Hydroxycut products, including serious liver injury, seizures, cardiovascular disorders, and rhabdomyolysis, a dangerous type of muscle damage. One death, due to liver failure, has also occurred.

Liver injury was previously listed as possible side effect of Hydroxycut. Symptoms of liver failure or liver dysfunction are jaundice, dark urine, nausea and vomiting, itching, loss of appetite, fatigue, and abdominal pain.

Hydroxycut is manufactured by Iovale Health Sciences, Inc. According to the company Web site, Iovale manufactures over 750 items that are sold in over 70 countries. The FDA warning affects only Hydroxycut products, which, according to Iovale, are taken by millions of consumers each year. Iovale has agreed to recall Hydroxycut products from the market.

Hydroxycut has raised doubt in the minds of consumers in the past. Its original formulation contained ephedra (ma huang), a substance that was banned by the FDA in April 2004 due to reported serious side effects and deaths associated with its use. Now the "new" formulation of Hydroxycut also appears to be unsafe. While the company's online information on Hydroxycut has been taken down due to the FDA warning, retailer sites indicate that the "new" (post-ephedra) formula is based upon a substance referred to as Hydroxagen, a blend of an extract from the plant *Garcinia cambogia* and other ingredients. The active ingredient in *Garcinia cambogia*, hydroxycitric acid (HCA), is claimed to have carbohydrate-blocking properties. Although a few studies have suggested a weight-loss benefit for *Garcinia cambogia* extract, the evidence is far from conclusive.

Weight-loss supplements, such as Hydroxycut, will remain on the market as long as there are millions of people looking for a "quick fix" or a way to "trick" their system into losing weight at an accelerated pace. But the long-term safety of these products has never been established, and their effectiveness has not been proven in independent, controlled clinical trials.

It may not be the answer everyone wants to hear, but the safest weight loss occurs by following a physician-supervised healthy eating and exercise plan. Those with significant obesity may want to discuss the use of an FDA-approved prescription weight-loss medication with their health-care professional. Meridia and Xenical are the only weight-loss drugs approved for longer-term use in significantly obese people, but even the safety and effectiveness of these products have not been established for use beyond two years. If you're taking Hydroxycut products (see the FDA advisory for the list of affected products), the FDA urges you to stop now and return them to the place of purchase. If you're taking Hydroxycut and believe you may have symptoms associated with its use, contact your health-care professional for an evaluation.

Reference: FDA news, May 1, 2009: http://www.fda.gov/bbs/topics/NEWS/2009/NEW02008.html

Last Editorial Review: 5/1/2009

Share | | | | | More

Also on MedicineNet
- Tips to Ease Nighttime Pain
- Type 2 Diabetes: Manage Your Symptoms

Kushner Attachment H

Popular Collections

all images »
all slideshows »

Scales, Plaques & Eruptions

Quitting Smoking

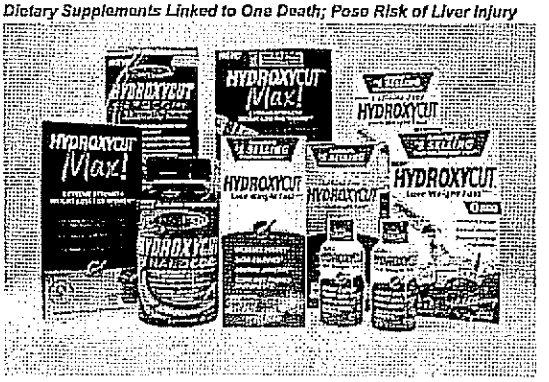
How to Avoid Weight Gain

MedicineNet.com

Source: Government

FDA Warns Consumers to Stop Using Hydroxycut Products

Dietary Supplements Linked to One Death; Pose Risk of Liver Injury



This picture shows some of the Hydroxycut products that are being recalled

The U.S. Food and Drug Administration is warning consumers to immediately stop using Hydroxycut products by Iovale Health Sciences Inc., of Oakville, Ontario and distributed by Iovale Health Sciences USA Inc. of Blasdell, N.Y. Some Hydroxycut products are associated with a number of serious liver injuries. Iovale has agreed to recall Hydroxycut products from the market.

The FDA has received 23 reports of serious health problems ranging from jaundice and elevated liver enzymes, an indicator of potential liver injury, to liver damage requiring liver transplant. One death due to liver failure has been reported to the FDA. Other health problems reported include seizures; cardiovascular disorders; and rhabdomyolysis, a type of muscle damage that can lead to other serious health problems such as kidney failure.

Liver injury, although rare, was reported by patients at the doses of Hydroxycut recommended on the bottle. Symptoms of liver injury include: jaundice (yellowing of the skin or whites of the eyes) and brown urine. Other symptoms include:

- nausea,
- vomiting,
- light-colored stools,
- excessive fatigue,
- weakness,
- stomach or abdominal pain,
- itching, and
- loss of appetite.

"The FDA urges consumers to discontinue use of Hydroxycut products in order to avoid any undue risk. Adverse events are rare, but exist. Consumers should consult a physician or other health care professional if they are experiencing symptoms possibly associated with these products," said Linda Katz, M.D., interim chief medical officer of the FDA's

Center for Food Safety and Applied Nutrition.

Hydroxycut products are dietary supplements that are marketed for weight-loss, as fat burners, as energy-enhancers, as low carb diet aids, and for water loss under the Iovate and MuscleTech brand names. The list of products being recalled by Iovate currently includes:

- Hydroxycut Regular Rapid Release Caplets
- Hydroxycut Caffeine-Free Rapid Release Caplets
- Hydroxycut Hardcore Liquid Caplets
- Hydroxycut Max Liquid Caplets
- Hydroxycut Regular Drink Packets
- Hydroxycut Caffeine-Free Drink Packets
- Hydroxycut Hardcore Drink Packets (Ignition Stix)
- Hydroxycut Max Drink Packets
- Hydroxycut Liquid Shots
- Hydroxycut Hardcore RTDs (Ready-to-Drink)
- Hydroxycut Max Aqua Shed
- Hydroxycut 24
- Hydroxycut Carb Control
- Hydroxycut Natural

Although the FDA has not received reports of serious liver-related adverse reactions for all Hydroxycut products, Iovate has agreed to recall all the products listed above. Hydroxycut Cleanse and Hoodia products are not affected by the recall. Consumers who have any of the products involved in the recall are advised to stop using them and to return them to the place of purchase. The agency has not yet determined which ingredients, dosages, or other health-related factors may be associated with risks related to these Hydroxycut products. The products contain a variety of ingredients and herbal extracts.

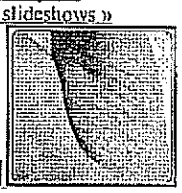
Health care professionals and consumers are encouraged to report serious adverse events (side effects) or product quality problems with the use of these products to the FDA's MedWatch Adverse Event Reporting program online, by regular mail, fax or phone.

FDA News Press Release May 1, 2009

Last Editorial Review: 5/1/2009

Popular Collections

all images »
all slideshows »



Scales, Plaques & Eruptions

Share | | | | | More

Quitting Smoking

How to Avoid Weight Gain

MedicineNet.com: